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Environmental Impacts to Stream Acidification and Brook Trout Populations in the Great Smoky Mountains National Park

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To the Graduate Council:

I am submitting herewith a dissertation written by Keil Jason Neff entitled "Environmental Impacts to Stream Acidification and Brook Trout Populations in the Great Smoky Mountains National Park." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Civil Engineering.

John S. Schwartz, Major Professor

We have read this dissertation and recommend its acceptance:

Robert B. Robinson, Theodore B. Henry, Qiang He, Glenn A. Tootle

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Dissertation
Presented for the
Doctor of Philosophy
Civil Engineering
The University of Tennessee, Knoxville

Keil Jason Neff
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Finally, I give thanks to this amazing and beautiful Earth, so full of life and energy. I hope we can keep "our home" clean and healthy, and live in balance with our natural environment. It has been a great privilege to have worked in the streams of the Great Smoky Mountains National Park!

Abstract

This research supports development of aquatic resource management strategies to address acid deposition in the Great Smoky Mountain National Park (GRSM) by 1) developing relationships between baseflow and stormflow chemical constituents and examining effects of elevation, area, geology, soil, and vegetation on stream chemistry; 2) evaluating physiological condition in brook trout in relation to changes in stream chemistry during stream acidification episodes, and 3) evaluating brook trout metrics with respect to stream chemistry, basin characteristics, and ecologically relevant hydrologic parameters. (1) Stream chemistry was monitored in eight GRSM streams considering basin area, site elevation, Anakeesta geology, soil, and vegetation. Following precipitation events, pH was significantly reduced and aluminum concentrations increased, while the concentration response of ANC, nitrate, sulfate, and base cations varied. Higher pH and ANC concentrations were observed in large and low-elevation streams. (2) Caged brook trout were exposed to two acid episodes during *in situ* bioassays conducted in three GRSM streams. Stream pH decreased (>0.7 pH units) and total dissolved aluminum increased (>0.175 mg/L) at all three sites during acid episodes. Whole-body sodium concentrations were significantly reduced (10-20%) when preceding 24-h time weighted average pH values (4.88, 5.09, 4.87) and corresponding 24-h aluminum concentrations (210, 202, 202 μg/L). Lower whole-body sodium concentrations were correlated with elevated proton and aluminum concentrations indicating physiological distress. (3) Water chemistry, hydrology and physical basin factors influenced brook trout distributions and densities in 16 collocated fish and water quality sampling sites (1990-2009). Higher concentrations of ANC, pH, sodium, and soil cation exchange capacity, and higher fall flows were associated with the presence of brook trout.

Trout densities were higher in streams with higher concentrations of sodium, suggesting that sodium may ameliorate the effects of acid toxicity. These relationships provide useful information where GRSM managers can prioritize conservation and restoration efforts.

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Chapter I: Introduction

In the Great Smoky Mountains National Park (GRSM), atmospheric acid deposition has impacted baseflow and stormflow water chemistry in some streams (Cai et al., 2009; Deyton et al., 2009; Robinson et al., 2008), potentially threatening brook trout (Neff et al., 2009) and other aquatic populations. The GRSM, located in the southern Appalachians in eastern Tennessee and western North Carolina (Figure 1), receives high rates of acid deposition in North America in the form of sulfur and nitrogen compounds (Baumgardner et al., 2003; NADP, 2009; Weathers et al., 2006), which contribute to the acidification of surface waters and soils (Driscoll et al., 2001). Although there have been reductions in acid deposition over the past two decades (NADP 2009), stream chemical response to decreased acid inputs has been limited spatially and temporally in the GRSM (Robinson et al., 2008) and other acid sensitive areas in the Eastern U.S.A. (Lawrence et al., 2008; Webb et al., 2004). Acids enter poorly buffered streams of the GRSM through wet deposition, and from accumulated dry deposition and naturally occurring organic acids flushed from watersheds during precipitation events (Cook et al., 1994; Deyton et al., 2009). Many of the streams and watersheds in the GRSM have low acid neutralizing capacities (ANC) and cannot buffer inputs from acid deposition. The consequent reduction in pH releases aluminum, which is highly toxic to many species of aquatic organisms (Driscoll et al., 2003). In 2006, 67-km of 12 streams in the GRSM were listed on the 303d list (Figure 2) as impaired due to low pH from atmospheric deposition and unknown sources (TDEC, 2006).

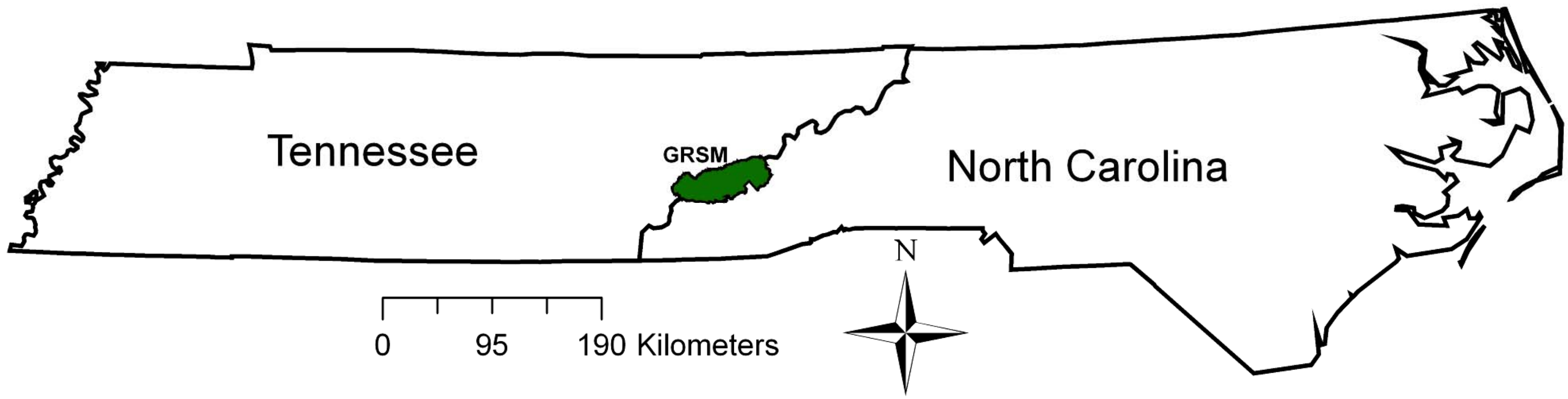

Figure 1: Location of the Great Smoky Mountains National Park.

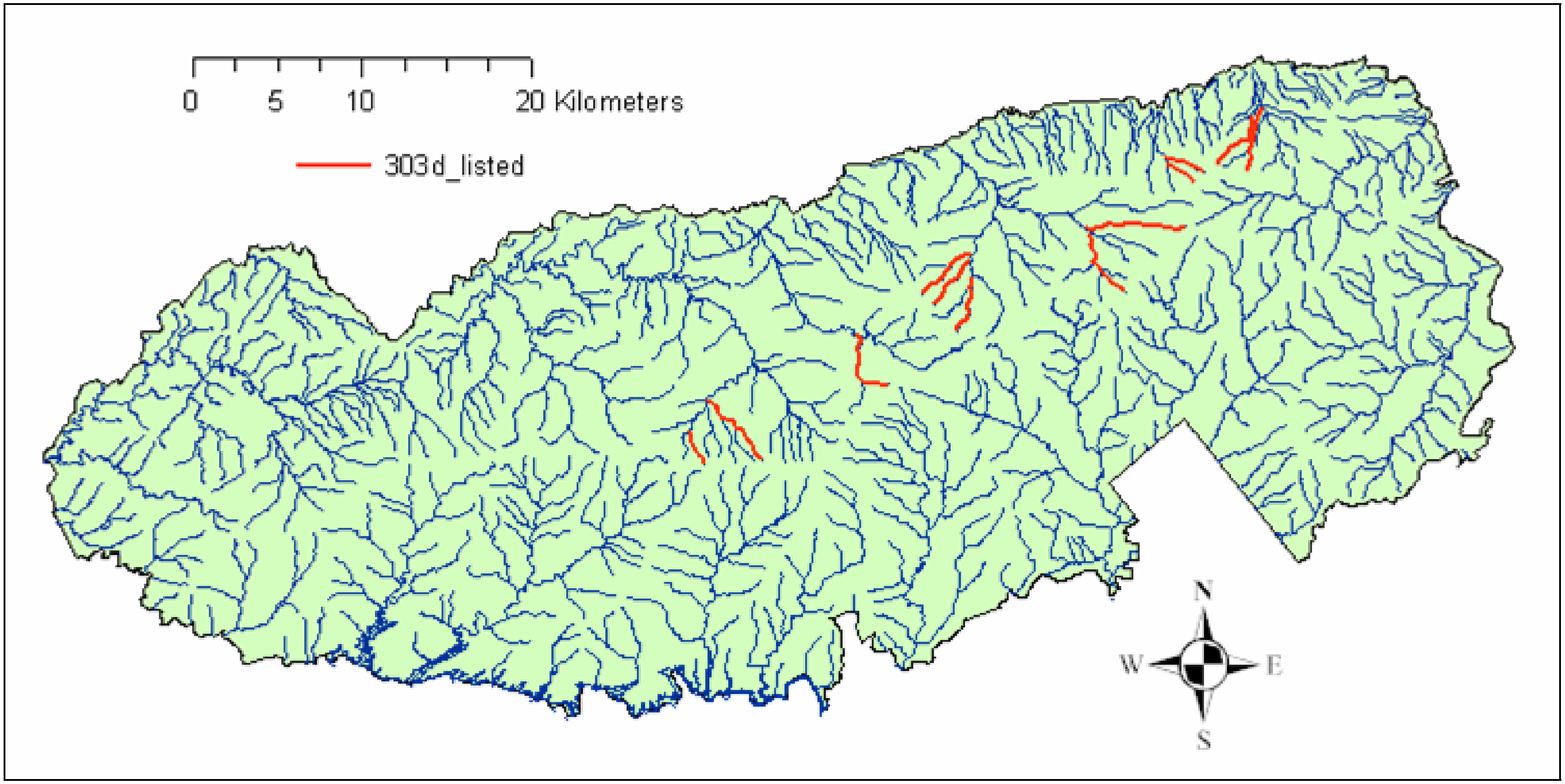

Figure 2: 303d listed streams in the Great Smoky Mountains National Park.

Although effects have not been observed directly, acidic episodes are suspected to be a potential cause of native brook trout (*Salvelinus fontinalis*) extirpation in some headwater streams in the GRSM, including some that were populated as recently as 15 years ago (Moore and Kulp, 2010). Fish can die or experience sublethal physiological stress when exposed to acid conditions (Baldigo et al., 2007; MacAvoy and Bulger, 2004; Woodward et al., 1991). The consequences of sublethal stress from acid episodes include downstream immigration (Gagen et al., 1993), reduced reproduction (Kaeser and Sharpe, 2001), impaired swimming (Wilson and Wood, 1992), and decreased growth (Cleveland et al., 1991; Mount et al., 1988). Episodes of stream acidification may cause native brook trout to migrate downstream, and physical barriers such as waterfalls and cascades limit recolonization in high-elevation headwater streams. In some GRSM streams, acid inputs have been shown to cause pH depressions of 0.5 to 2.0 units (to as low as pH of 4.1) during stormflows (Cook et al., 1994; Deyton et al., 2009). Based on long term declines in stream pH (Robinson et al., 2008), GRSM resource managers fear that brook trout may continue to be impacted by stream acidification.

A conceptual model of acid contributions to GRSM basins and streams has been developed in multiple discussions between GRSM personnel and The University of Tennessee, Knoxville (UTK) faculty and students, including GRSM biologists Steve Moore and Matt Kulp, and UTK researches John Schwartz, Bruce Robinson, Ted Henry, and Keil Neff. Figure 3 illustrates further development of this model. Landscape topography affects the local climate including temperature, humidity, cloud cover, wind speed, and orographic rain. Slope is also a primary driver of the hydrology and hydraulics of surface water. Topography, geology, and geomorphology control the

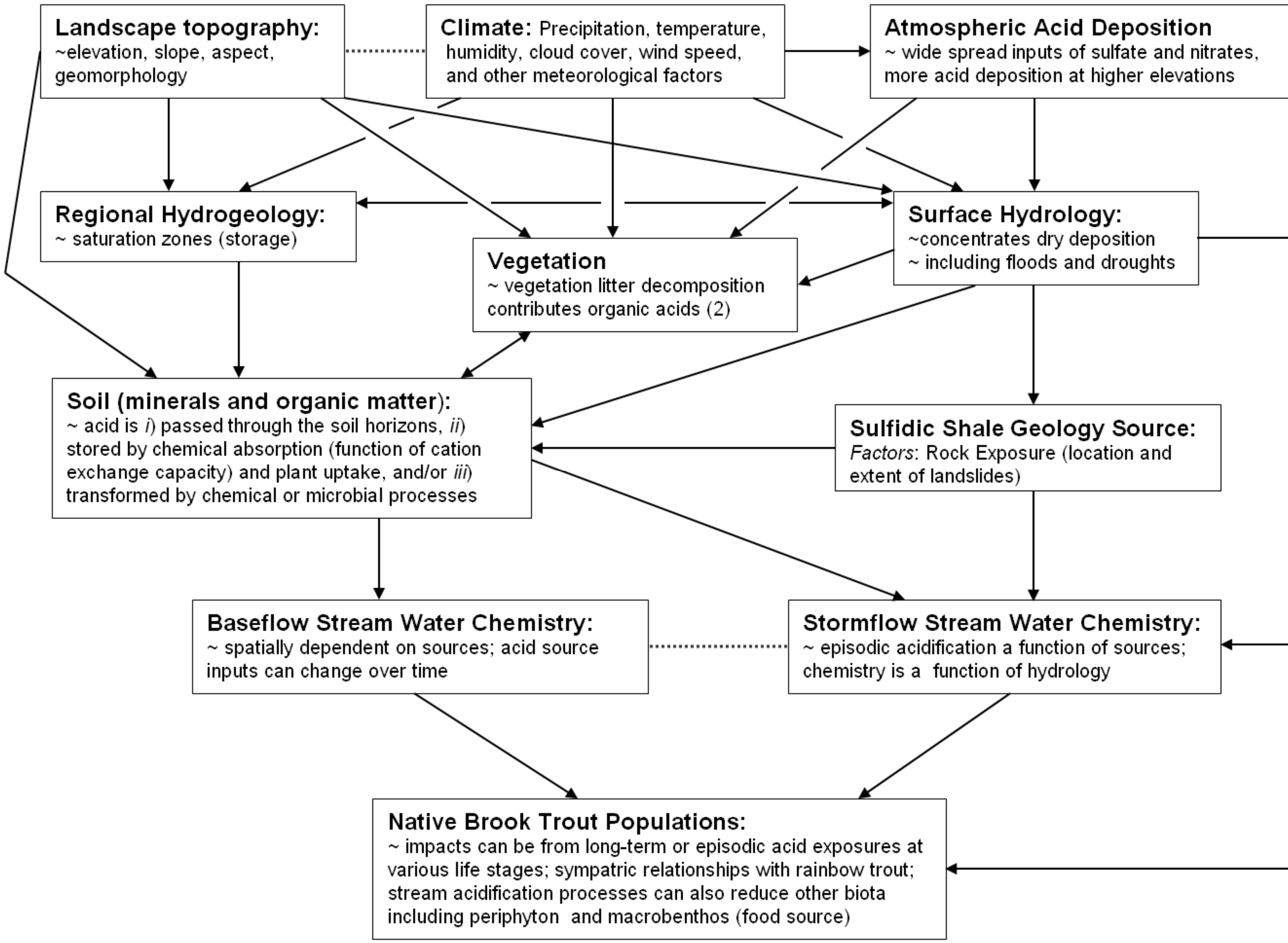

Figure 3: Conceptual model of acid contributions to GRSM basins.

interaction between precipitation (including atmospheric acid inputs) and stream water quality; soil and vegetation act as system filters between hydrological and hydrochemical components (Billett and Cresser, 1992; Mulholland, 2004). Water is routed through both surface and subsurface flow paths (McGuire et al., 2005), or is returned to the atmosphere through evapotranspiration. Constituents of acid deposition are transported through, used by, or stored in surface flow, vegetation and/or upper horizons of soil (Andersson and Nyberg, 2009). Additional acid sources include organic acids (Driscoll et al., 1989) and disturbed Anakeesta in contact with air and water (Bacon and Maas, 1979). Water and acid constituents continue through the system in baseflow and stormflow. Baseflow and stormflow are related as i) baseflow is a component of stormflow and ii) because of their spatial linkage (i.e. the same geochemical and physiographic characteristics influence the water quality in any particular stream location). Water quality, in the aquatic environment of brook trout and other aquatic organisms, affects complex biotic processes and relationships.

It is imperative to understand the watershed-scale processes associated with stream acidification, determine system responses to atmospheric deposition, and evaluate potential impacts to lotic biota. The research presented in this dissertation supports the management of aquatic resources from potential acid deposition impairment in the GRSM by 1) developing relationships between baseflow and stormflow chemical constituents and examining the effects of elevation, area, geology, soil, and vegetation on stream chemistry; 2) evaluating changes in physiological condition in brook trout in relation to changes in stream chemistry during episodes of stream acidification, and 3) evaluating brook trout metrics with respect to stream chemistry, basin characteristics, and ecologically relevant hydrologic parameters.

This dissertation advances the understanding of acidification response as influenced by system drivers and filters (basin characteristics), and how native brook trout are affected by hydrochemistry. Chapter III is unique in evaluating the influence of basin factors on stormflow stream chemistry in addition to baseflow chemistry, thereby expanding the range for spatial assessment of potential impacts from episodic acidification. Episodic acidification was directly linked to sublethal physiological distress in southern strain brook trout from *in situ* experimentation in Chapter IV. In the final study (Chapter V), factors influencing the density and health of brook trout, including water quality and hydrological extremes (as functions of basin characteristics) were identified. This is unique from other studies such that multiple factors (chemical, hydrological and basin) were evaluated concurrently to understand how the interaction of these factors influence trout density and health, and to determine which factors explained the greatest proportion of variability within populations.

Research Objectives

The following objectives, and testable hypotheses, were integrated with new experimentation and established long-term monitoring projects.

I. Characterization of stream chemistry in distinct GRSM basins

Objective I.1: Develop relationships between baseflow and stormflow chemical constituents.

Hypothesis I.1.1: Streams with lower baseflow ANC will have greater decreases in stream pH during stormflows.

Hypothesis I.1.2: Streams with elevated dissolved metals (i.e., Al, Cu, and Zn) during baseflow will have higher concentrations of these metals during acid episodes.

Objective I.2: Examine the effects of elevation, basin area, and Anakeesta geology on stream chemical response to acidification.

Hypothesis I.2.1: Streams in higher elevation basins will have lower baseflow and stormflow pH and ANC concentrations, and higher sulfate and nitrate concentrations.

Hypothesis I.2.2: Streams in larger basins will have higher concentrations of ANC base ions in stream chemistry and will be able to buffer acid inputs from precipitation events to a greater extent than streams in smaller basins.

Hypothesis I.2.3: Streams in basins with Anakeesta geology will have lower pH, and sulfate and aluminum concentrations than streams in basins without Anakeesta geology.

Objective I.3: Investigate the influence of physical and chemical soil properties and dominant vegetation on stream chemistry.

Hypothesis I.2.1: Streams in basins with lower soil pH will be more acidified than streams in basins with higher soil pH.

Hypothesis I.2.2: Dominant vegetation types will be significantly correlated with stream chemical constituents in baseflow and stormflow.

II. *In situ* toxicity bioassays

Objective II.1: Evaluate changes in physiological condition in wild southern strain brook trout before, during, and after *in situ* episodes of stream acidification and relate these changes to differences in stream chemistry.

Hypothesis II.1: During periods of acute stream acidification from acidic deposition in the GRSM, native brook trout lose sodium in a manner that is consistent with physiological distress associated with low pH and elevated Al concentrations.

III. Trout population relationships with chemical, hydrological and basin factors

Objective III.1: Determine factors affecting native brook trout density and distribution in relation to i) baseflow and stormflow stream chemistry, ii) physical watershed characteristics, and iii) hydrologic condition.

Hypothesis III.1.1: Significant factors promoting brook trout populations include i) higher elevations, ii) small basin areas, iii) lower depositional rates, iv) higher stream pH and ANC concentrations, and v) larger fall flows.

Hypothesis III.1.2: Significant factors limiting brook trout populations include i) presence of Anakeesta within basins, ii) elevated sulfate and dissolved organic carbon (DOC) concentrations, and iii) hydrologic disturbances (floods and droughts).

Objective III.2: Assess the relative importance of the chemical, basin, and hydrology variables to annual variation in brook trout densities.

Hypothesis III.2.1: Chemical factors, particularly pH and aluminum concentrations, will be the dominant control of brook trout distribution, and will explain the variation in density of brook trout to a greater extent than the other factors.

Study Area

The GRSM is located in the Blue Ridge physiographic region of the southern Appalachians in eastern Tennessee and western North Carolina. This physiographic region is characterized by rugged topography, heavily forested slopes, and steep mountain streams which flow into the Tennessee River system. Altitudes in the GRSM range from 300 m to 2,025 m. GRSM watersheds are characterized by steep gradients and thin sandy loams that provide poor buffering capacities. Streambeds are dominated by boulder and cobble, and gradients of the stream channels increase with elevation (Larson and Moore, 1985). Approximately 80% of the GRSM is comprised of deciduous forests including more than 1,300 species of flowering plants and 130 native trees (Whittaker, 1956). At elevations greater than 1,500 m, red spruce - Fraser fir forests dominate. Hemlock forests dominate stream sides and moist, shady slopes up to 1,200 m and are currently threatened by the hemlock woolly adelgid (Ford and Vose, 2007).

The climate of GRSM is perhumid mesothermal with seasonal temperature variation and precipitation distributed throughout the year (Busing, 2005). The average annual rainfall varies significantly throughout the park with lower elevations generally receiving near 127 cm and some higher elevation sites near 216 cm (Busing, 2005). Most summer rainfall occurs during thunderstorms and an occasional tropical storm. Winter precipitation is associated with large-scale frontal systems. Summer and early spring generally have the most abundant precipitation, with rainfall averages of 12.7 cm and 20.3 cm per month in lower and upper elevations, respectively. Autumn is the driest season due to slow-moving high pressure systems, with rainfall averages around 7.6 cm and 12.7 cm per month in lower and upper elevations, respectively. Gatlinburg, TN (elevation = 486 m), just north (central) of the GRSM, has an average annual temperature near 14 °C; Clingmans Dome, the highest point in the GRSM, has an average annual temperature of 6°C (NOAA, 2002).

Long-Term Inventory & Monitoring Projects[1]

Two core datasets have led to the present hypotheses and development of this research and can be divided into long-term assessments of both abiotic and biotic variables that describe water quality and fish fauna conditions. These datasets are

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[1] Much of this section is from the introduction of the 2007 Annual Report of which Meijun Cai and I were the primary authors. Schwartz, J.S., Cai, M., Neff K.J., Zimmerman, G.T., Parker, J. Great Smoky Mountain Water Quality Annual Report for 2007. Report prepared for the National Park Service; Cooperative Agreement No.: 1443-CA-5460-98-006; Southern Appalachian Cooperative Ecosystem Studies Unit CESU Host Cooperative Agreement No. H5000 04 5040; Task Agreement No. J5460 05 0067; April 2008. It is included in this dissertation because (i) the core data sets were integral in the research, and (ii) to have this information regarding these I&M program components accessible to the public.

components of the Inventory and Monitoring (I&M) program of the National Park Service. To effectively manage resources amid environmental change, the NPS sponsored the I&M program, in which National Parks host research "living laboratories". Through this program, the NPS monitors important resources to provide early signs of impairment, thereby enabling resource managers to swiftly implement strategies to protect ecological integrity.

Two components of the I&M program discussed below include the GRSM steam survey and the fish sampling program. The data sets from these programs have been compiled by the NPS and UTK to answer both specific questions and identify long-term trends. Data from the stream survey and fish sampling program will be included in analyses and models of this dissertation.

Long-term synoptic baseflow stream water quality monitoring (park-wide stream survey) began in October of 1993 to identify the potential impacts of acid deposition on GRSM streams and monitor changes in stream acidification. Sites were selected to assess the spatial variability of water quality within the GRSM (as a whole and within particular watersheds) across a range of elevations, geology types, and disturbance histories. From 1993-1995, samples were collected at 367 sites semi-annually. In 1995, the number of sites was reduced to 160 and collected on a monthly basis; in 1997, the number of sites was reduced to 90 and collected quarterly (Figure 4). The number of sites was further reduced in 2004 to 32 sites sampled bimonthly and 11 sites sampled biannually in the Hazel Creek watershed (Figure 4). From October 1993 to December 1998, personnel in the Department of Forestry, Wildlife, and Fisheries at the UTK, performed chemical analyses of all samples collected in the stream survey program. From January 1998 to

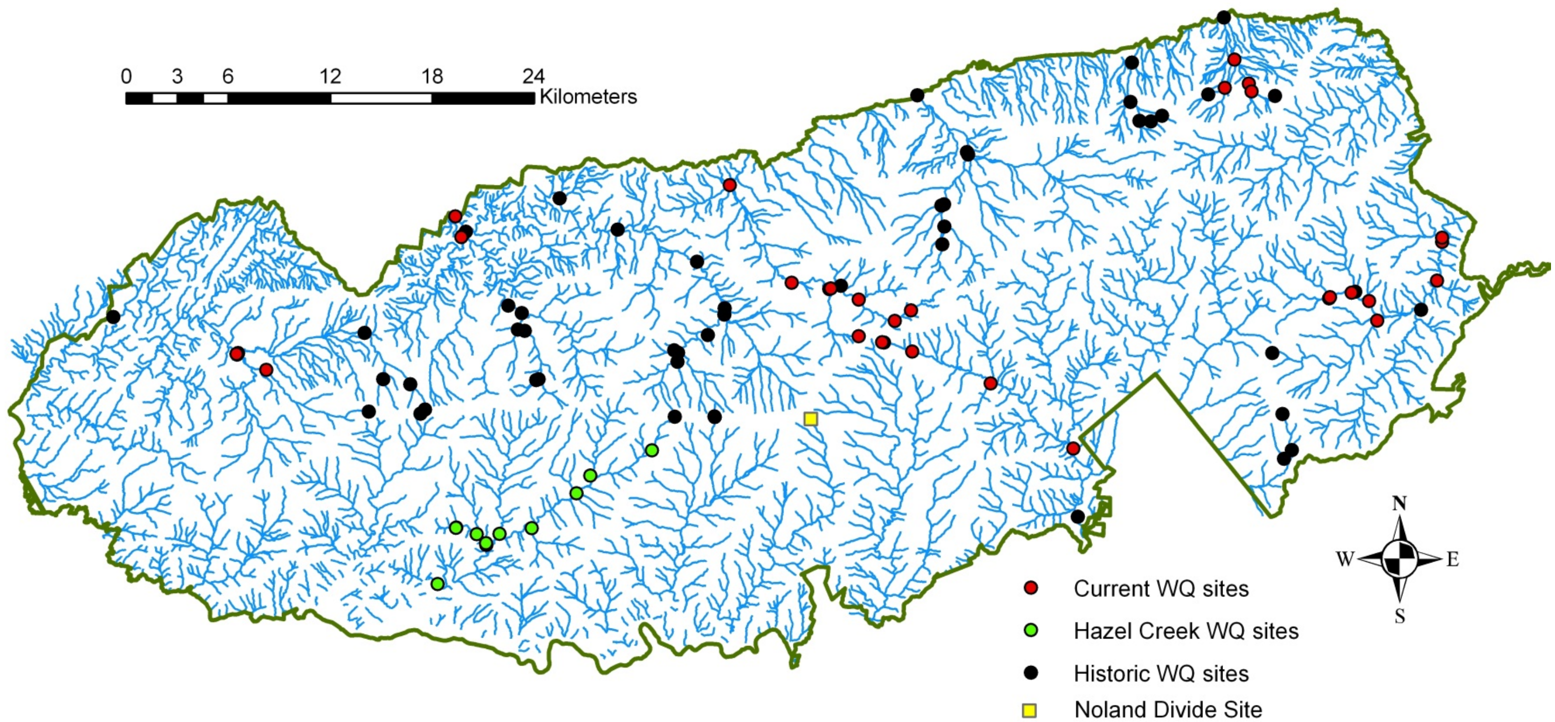

Figure 4: Baseflow water quality monitoring sites.

the present, staff and students in the Department of Civil and Environmental Engineering have performed all chemical analyses. The water quality parameters currently measured are pH, ANC, conductivity, sulfate, nitrate, chloride, ammonium, sodium, potassium, calcium, magnesium, aluminum, zinc, copper, iron, manganese, and silica.

Fish sampling in the GRSM has been conducted since the early 20th century and the current sampling program was established in the early 1980's. In 1993, the fish sampling program became part of the I&M program. Streams are sampled by GRSM fisheries biologists with standard multiple pass electroshocking. Assessments for trout species include detailed information on fish condition (length, weight), abundance, year class strength, biomass, and density. The 2007 trout distributions in the GRSM and fish sampling sites are illustrated in Figure 5.

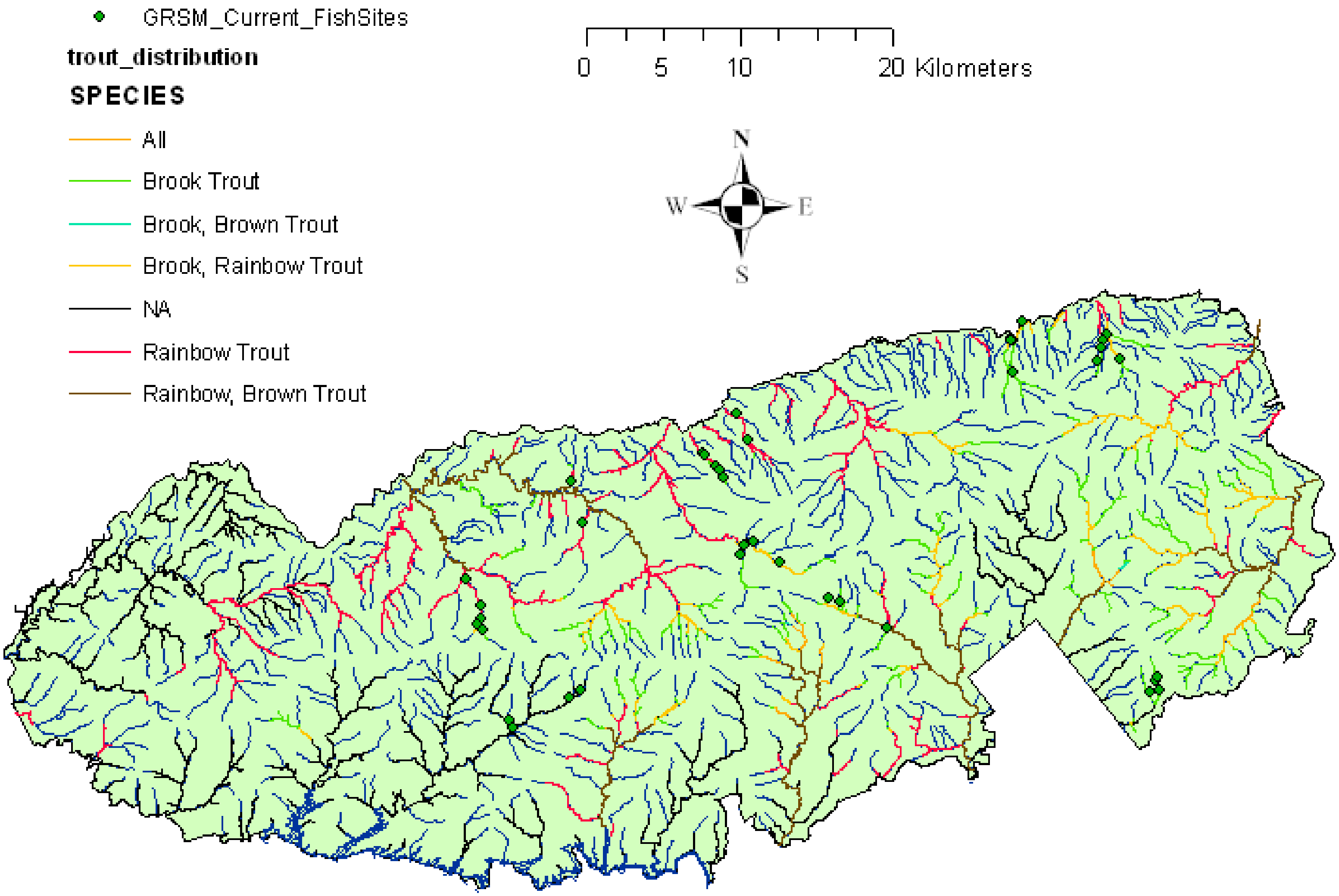

Figure 5: 2007 trout distribution and fish sites in the Great Smoky Mountains National Park.

Chapter II: Literature Review

GRSM Trout

Brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*) inhabit GRSM streams. Brook trout are the only salmonid species indigenous to the southern Appalachian Mountains (Warren et al., 2000). Rainbow trout were introduced into streams of the GRSM in the early 1900's and often live sympatrically with native brook trout (Larson et al., 1995). Rainbow trout usually dominate lower elevation streams; whereas, brook trout are predominantly found in headwaters. The distribution of the native brook trout populations has seen a general decline since 1900 (Larson and Moore, 1985). The population decline can be attributed to extensive logging and heavy fishing pressure in the first half of the 20th century, and to the advancement and success of rainbow trout in later years (Lohr and West, 1992; Whitworth and Strange, 1983). Brown trout, introduced in the late 1800's, are generally found in larger lower elevation streams and rivers, and often live sympatrically with rainbow trout (Bivens et al., 1985; Etnier and Starnes, 1993).

Environmental factors may limit the distribution of trout species and affect the composition of fish communities in the GRSM. Fish respond to an array of natural and anthropogenic factors including: stream chemistry, chemical inputs, physical habitat and hydrologic characteristics, recruitment success, fishing and stocking rates, competition, predation, and availability and quality of food resources (Baldigo and Lawrence, 2001).

Environmental Factors Affecting Trout

Acidification

The GRSM receives high rates of atmospheric acid deposition in the form of sulfur and nitrogen compounds (NADP, 2009), which contribute to the acidification of surface waters and soils (Driscoll et al., 2003). Atmospheric sulfur and nitrogen compounds, largely attributed to the burning of fossil fuels, are oxidized into sulfuric and nitric acids. National environmental regulations, including the 1990 Amendments to the United States Clean Air Act, and improvements in scrubber technologies, have reduced acidic deposition and supported some surface water recovery in North America (NADP, 2009; Warby et al., 2008). However, long-term atmospheric deposition can result in accumulation of sulfate, nitrate, ammonium, and proton (Wigington et al., 1996b). The effects of acid deposition include soil leaching of base cations and aluminum, and depressed stream pH and ANC (Driscoll et al., 2003). Wet deposition of sulfate, nitrate, and proton has been shown to increase at higher elevations because of higher rainfall associated with orographic precipitation (Baumgardner et al., 2003). Cloud water deposition can be significant at high elevations in the GRSM, but is highly variable due to wind speed, cloud structure, and canopy type (Weathers et al., 2006).

Acid deposition has been dominated by sulfur deposition in the past, but recent research shows the growing importance of nitrogen deposition (Sullivan et al., 2004). Nitrate mobility is determined by biological controls in addition to deposition rates and soil properties (Webb et al., 2004). Stream nitrate concentrations are lowest in the summer due to the uptake of nitrogen by vegetation. During the "leaf off" season, stream nitrate increases due to increased flow and decreased plant uptake (Cai et al., 2009). Atmospheric deposition of ammonium (NH_4) is derived from emissions of ammonia (NH_3), which result from automobiles and industrial processes (Driscoll et al., 2001). Ammonium can contribute to the acidification of soil and water if it is oxidized by soil microbes into nitrate.

The degree of stream acidification can be classified by a number of parameters including ANC, pH, and aluminum concentration. The most widely accepted measure of the degree of acidification is defined by Wigington et al. (1996a): i) chronically acidic: ANC < 0 μeq/L, ii) episodically acidic: 0 < ANC < 20 μeq/L, iii) transitional: 20 < ANC < 50 μeq/L, and iv) not acidic: ANC > 50 μeq/L. ANC is calculated as the total alkalinity of an unfiltered water sample. ANC is dependent on the acid-base chemistry and total carbonate concentration of the water. ANC in acidic waters may be calculated based on the concentration of bicarbonate (HCO_3^-) and proton (H^+) or by manipulation of the ion balance (μeq/L):

$$ANC = [HCO_3^-] - [H^+]$$

where:

or:

$$ANC = \sum C_B - [SO_4^{2-}] - [NO_3^-] - [Cl^-]$$

(Deyton et al., 2009; Hyer et al., 1995; Molot et al., 1989).

Many lakes and streams examined in a National Surface Water Survey (NSWS) suffer from chronic acidity, a condition in which water has a constant low pH level (Herlihy et al., 1991). Although chronic stream acidification from acid deposition occurs in some streams in the GRSM (Robinson et al., 2008), episodic acidification is of critical concern in the GRSM because of acute toxicity to trout (Neff et al., 2009). Acid episodes refer to reductions of acid neutralizing capacity (ANC), accompanied by increased concentrations of H^+ and Al; when ANC ≤ 0, these are termed acidic episodes (Wigington et al., 1996a).

Baseflow stream water originates from groundwater flow in the lower inorganic soil profile and has more time to interact with base cations. Stormflow is routed through the upper layer of the soil, which is generally more acidic due to acid deposition and organic processes. Stormwater has less time to react with base cations in the soil and is generally more acidic upon delivery to surface waters (Wigington et al., 1996b). Soil macropores may also play an important role in shallow groundwater flow because they allow acidic rain water to reach the stream quickly, thus minimizing the potential for acid buffering (Potter et al., 1988). As a result of these processes, baseflow water has higher ANC concentrations than stormflow water in poorly buffered streams of the GRSM (Deyton et al., 2009).

Robinson et al. (2008) performed step-wise multiple linear regression models to analyze baseflow pH, ANC, sulfate and nitrate long-term time trends. In this study, the potential predictor variables included cumulative Julian day, seasonality, elevation, basin slope, stream order, precipitation, surrogate stream flows, geology, and acid depositional fluxes (Robinson et al. 2008). Modeling revealed statistically significant decreasing trends in pH and sulfate with time at lower elevations, but generally no long-term time trends in stream nitrate or ANC (Robinson et al. 2008). If conditions remain the same and past trends continue, the forecasting models suggest that 30.0 % of the sampling sites will reach pH values less than 6.0 in less than 10 years, 63.3 % in less than 25 years, and 96.7 % in less than 50 years (Robinson et al. 2008).

Short term acidification processes in streams are associated with (1) increased concentrations of sulfate and nitrate from acid deposition, (2) the mobilization of organic acids, (3) increased acidity due to pyritic geology oxidation, and/or (4) the dilution of base cations caused by high stream flow (Deyton et al., 2009; Kahl et al., 1992; Lawrence, 2002; Tranter et al., 1994). Acid episodes do not occur solely from cation dilution; rather, base cation dilution in addition to increased acidic inputs causes episodic acidification. ANC losses are generally the result of a combination of the two processes, where acid-base reactions are more dominant than base-cation dilution (Kahl et al., 1992). The solubility of aluminum increases as pH decreases and is leached from soils in contact with low pH water (Cronan and Schofield, 1990). Once mobilized, aluminum can complex with organic and inorganic species or it may remain as free aluminum. At low pH values (<4.0), the majority of the aluminum is present as free aluminum (Al^{3+}) and as the pH increases, the free aluminum complexes with inorganic ligands such as hydroxide, fluoride, sulfate, etc. and organic species including humic acids (Warby et al., 2008).

In addition to decreased ANC concentrations, streams can be acidified by organic acids flushed from the upper soil horizons during storm events. The influence of organic materials can be quantified by measuring the DOC concentration in stream flow (Herlihy et al., 1991). Waters with high concentrations of DOC can be acidic due to their organic acid content, as approximately 50% of DOC is comprised of humic substances including humic acids, fulvic acids, and humin (Eaton et al., 2005). Streams with DOC concentrations of 1-10 mg/L can be considered “organic influenced”; streams with organic anion concentrations greater than the sum of sulfate and nitrate concentrations would be considered "organic dominated" (Herlihy et al., 1991). Cook et al. (1994) found that DOC levels increased by 200 μmol/L in a spring stormflow in the GRSM; this increase in organic acids probably contributed to a pH drop of one unit. Stormflow routed through shallow soil transports organic acids from the organic-soil horizons; baseflow flows through the lower mineral soil which tends to absorb DOC (Cook et al., 1994).

Deyton et al. (2009) determined stream acidification may be driven by acid deposition in the GRSM, but additional inputs from varying vegetation and geology create unique and complex responses to the observed acidification. During stormflow, ANC and pH depressions were observed for all storms at three study sites in the Middle Prong of the Little Pigeon River watershed. Sulfate, nitrate, and organic acid concentrations increased during acid episodes. ANC contribution analysis indicated acid deposition was the primary cause of episodic acidification; however, organic acids and cation dilution also contributed to acidification of streams, albeit to a lesser extent. In addition, large storms preceded by long, dry periods caused the largest pH depressions.

Streams can also be acidified by natural or anthropogenic disturbances of Anakeesta geological formations. Anakeesta is a carbonaceous phyllite which is known to produce elevated concentrations of aluminum, manganese, and zinc when disturbed, and is a potentially significant source of acidification for surface waters (Huckabee et al., 1975). Anakeesta is relatively non-reactive until exposed to air and water, whereupon it oxidizes to release acid and heavy metals to adjacent bodies of water. Sulfate is generally the highest concentrated anion in streams contaminated by Anakeesta oxidation (Minear and Tschantz, 1976). The Anakeesta Formation, commonly found in high elevations in the GRSM (Figure 6), is comprised of carbonaceous and sulfidic slate or mica schist, as well as varying quantities of sulfide (Bacon and Maas, 1979). During the 1963 construction of US 441 in the GRSM, Anakeesta formations were disturbed which resulted in the elimination of aquatic species in Beech Flats Creek (Bacon and Maas, 1979; Kucken et al., 1994). Likewise, streams were "rendered virtually lifeless" from natural Anakeesta disturbances in Alum Cave Creek and Walker Camp Prong watersheds (Bacon and Maas, 1979).

Mechanisms of Acid Toxicity

Fish can die or experience sublethal physiological stress when exposed to acid conditions (Baldigo et al., 2007; MacAvoy and Bulger, 2004; Woodward et al., 1991). The duration and severity of acid episodes are the most important factors influencing fish survival (Van Sickle et al., 1996). The mechanism of acid stress in fish is generally recognized as a disturbance of ion regulation that can lead to circulatory collapse and ultimately death (Booth et al., 1988). Increased H^+ concentrations (low pH) interfere with gill ion transport systems and diminish influx and greatly increase efflux of sodium (Grippo and Dunson, 1996). A rapid drop in plasma sodium by more than 30% triggers a reduction in plasma volume due to water, thereby increasing blood viscosity which elevates arterial blood pressure facilitating circulatory failure resulting in death from hypoxia (Milligan and Wood, 1982). In addition to low pH, the concentrations of monomeric inorganic aluminum (Al_{IM}) and calcium impact ion regulatory mechanisms

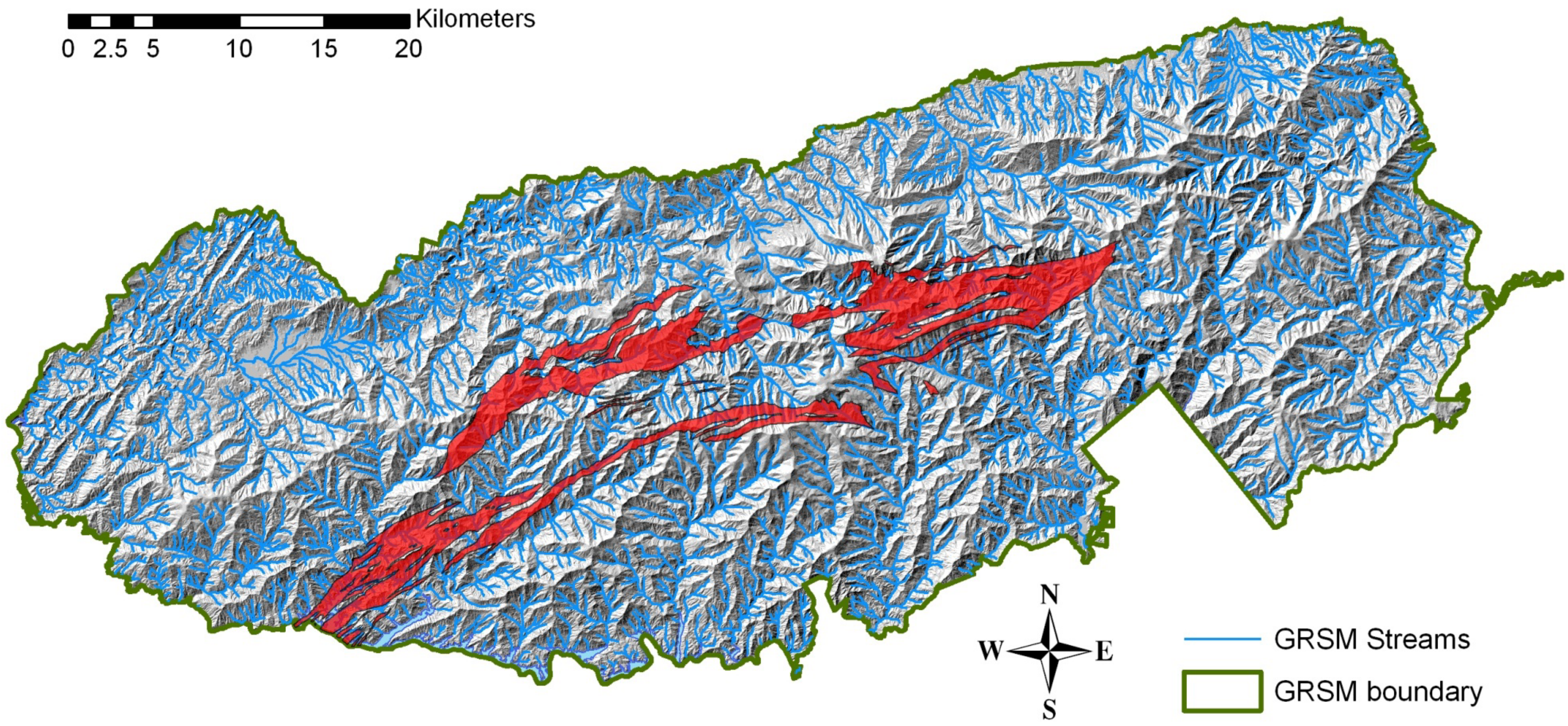

Figure 6: Anakeesta and Copperhill formations (sulfidic shale) in the GRSM.

(Baker and Schofield, 1982). Aqueous aluminum is mobilized from the edaphic to aquatic phase during episodes of acidification (Driscoll et al., 1980). Inorganic fractions of aluminum increase with decreases in pH and predominate in waters below pH 5.0 (Driscoll et al., 1995; Sullivan and Cosby, 1998). Like H^+ toxicity, the primary mechanism of Al_{IM} toxicity is a disturbance of gill ion transport (Booth et al., 1988) when pH is 4.2 to 4.8 (most severe at pH = 4.5), and asphyxia when pH is 5.5 to 6.4 (most severe at pH = 6.1), (Neville and Campbell, 1988). Toxic metal ions, including Al_{IM}, compete with metal cations (particularly Ca^{2+}) on channel proteins in the gill surface (Di Toro et al., 2001) and facilitate greater loss of critical blood ions (Wood et al., 1990). In waters of low hardness, less dissolved Ca^{2+} is available and fish are more vulnerable to loss of ions induced by elevated Al_{IM} and H^+ (Cleveland et al., 1991; Ingersoll et al., 1990).

Episodic acidification events generate water chemistry conditions that are different than those observed in persistent low pH waters and these conditions have different consequences on fish physiology. During an acidification event, stream pH can change rapidly and cause disequilibrium conditions to exist for many metal ions including Al. Inorganic fractions of aluminum, mobilized when stream pH decreases, are toxic to fish (Driscoll et al., 1980; Exley et al., 1991; Gensemer and Playle, 1999). Under rapidly increasing pH, aluminum polymerization can occur, both in stream water and within the gill microenvironment, which can result in acute toxicity (Poleo, 1995). The consequences of episodic acidification events on fish are dependent on the extent of the change in pH, the duration of the event, and the time interval between successive acid episodes (Calta, 2002; Gagen et al., 1993).

Surface water concentrations of inorganic monomeric aluminum are highly related to pH while organic monomeric aluminum concentrations are highly related to DOC concentrations (Driscoll et al., 2001). For salmonids, DOC has an ameliorating effect on the toxicity of aluminum (McCartney et al., 2003). When present in stream water, DOC complexes with inorganic aluminum to create organic monomeric aluminum, which is less toxic to fish. While studying fresh water streams in Scotland, McCartney et al. (2003) noticed a nearly two-fold decline in the toxic form of aluminum when DOC was significantly increased. Similarly, in a study on brook trout in the Catskill Mountains of New York, Baldigo and Murdoch (1997) observed that DOC concentrations greater than 2 or 3 mg/l may significantly decrease the concentration of Al_{IM}. The ameliorating effects of DOC were most pronounced when concentrations were greater than 3.1 mg/l and water pH was between 5.2 and 5.6 (Baldigo and Murdoch, 1997).

For fish in aquatic environments, external calcium and internal levels of the hormone prolactin combine to control the permeability of the gill (Hunn, 1985). The presence of calcium in water reduces the permeability of fish gills to both H^+ and sodium. Hesthagen et al. (1999) found the threshold value at which calcium reduces gill membrane permeability in Norway brown trout is 1.0 mg/L. Additionally, calcium competes for interaction sites with toxic metal species, and therefore ameliorates the toxic effects of Al_{IM} and H^+. In soft acid waters, 3 mg/L of calcium can reduce the toxicity of aluminum to fish (Hunn, 1985).

Jackson (2006) applied water quality data to determine relationships between trout biomass and base flow water quality in the GRSM. Final correlation analysis, where zeros were assumed for biomass when there were no trout present, revealed that pH, ANC, conductivity, and sulfate were important predictors of trout biomass (Jackson, 2006). Trout species were negatively affected by increases in sulfate concentrations and percent Anakeesta geology (Jackson, 2006). Rainbow trout biomass was found to increase with increasing stream conductivity and ANC (Jackson, 2006). Negative correlations between sulfate concentrations and biomass provided circumstantial evidence that acid deposition impacts trout populations in the GRSM.

Consequences of Sublethal Acid Toxicity

The duration, magnitude, chemical composition, timing and spatial distribution of acid events influences the survival and stress response of trout (Baldigo and Murdoch, 1997). The result of sublethal sodium loss in trout from acid episodes include downstream immigration (Gagen et al., 1994), less successful reproduction (Kaeser and Sharpe, 2001), impaired swimming performance (Wilson and Wood, 1992), and decreased growth (Cleveland et al., 1991; Mount et al., 1988).

Episodes of stream acidification may cause native brook trout to migrate downstream, and physical barriers such as waterfalls and cascades limit recolonization in high-elevation headwater streams. Significant downstream movements of brook and brown trout to avoid low pH conditions brought on by acid episodes were documented in Linn Run in southwestern Pennsylvania (Carline et al., 1992b; Gagen and Sharpe, 1987). Carline et al. (1992a) speculate adult survival from acid episodes is dependent on watershed and channel morphological conditions that form chemical refugia from low pH during stormflows. Unless local areas of groundwater upwelling are present to provide refuge, fish often respond to acid episodes by emigrating downstream (Kocovsky and Carline, 2005). However, if chemical refugia does not exist, adult brook trout would spend more energy moving downstream to higher pH waters, followed by upstream return to occupy headwater reaches without food competition from rainbow trout in lower watershed reaches (Larson and Moore, 1985). Poor energetics would reduce fish fecundity over time depending on watershed condition.

Acute acid episodes, more pronounced in high elevation watersheds in the GRSM, principally affect greater number of fish than chronic exposures (Allin and Wilson, 2000). Trout populations in higher elevation streams may be at greater risk to acid episodes than trout in streams at lower elevation. Native brook trout inhabit 55 km of GRSM streams below 914 m (GRSM fish database). Above 914 m, there are 255 km of brook trout streams to elevations exceeding 1500 m (GRSM fish database). Understanding the ecological processes that lead to brook trout extirpation in some watersheds but not in others is complex, which requires knowledge of: 1) spatial and temporal patterns of episodic stream acidification related to watershed basin area and elevation, climate, geology, soils, and vegetation; 2) life history patterns specifically related to spawning, fry emergence, summer rearing, and home range; and 3) toxicological sensitivity to episodic exposures (Al_{IM}, H^+) and dose-frequency response of trout related to life stage.

Sensitivity, Growth, and Reproduction

Brook trout, native to eastern North America and more tolerant to acidic conditions than brown or rainbow trout (Kocovsky and Carline, 2005), are most often regulated to small lakes and streams at high elevations, which are most susceptible to acid deposition (Turner et al., 1992). As with most fish, early life stages of brook trout are more acid sensitive than the older ones (Baldigo and Lawrence, 2001) and exhibit higher sodium turnover rates (Grosell et al., 2002). Trout species may have decreased spawning habitat to avoid low pH, which can lead to reduced egg production and survival (Curry and Noakes, 1995). Brook trout avoided waters of pH 4.5 for spawning, and streams with pH 4.8 and adequate spawning gravels were unable to support trout populations (Curry et al., 1994). It has been demonstrated that brook trout can select areas of alkaline upwellings from groundwater in which to build their redds (Curry and Noakes, 1995).

Toxicological studies found measurable reductions in growth and survival of brook trout at aluminum concentrations of 0.2 mg/l and a pH of 5.0 (Gagen and Sharpe, 1987). Acidity was a nonlethal inhibitor of growth of brook trout at pH levels of 5 to 6 (Baldigo and Lawrence, 2001). Reduced body size of fish from acid stress has been shown to cause reduced egg production (Harvey and Jackson, 1995; Mount et al., 1988). The reduction of deposited eggs or the inability to lay eggs by mature adults subjected to chronic low pH has been documented in salmonid species (Baker and Schofield, 1982; Mount et al., 1988). In rainbow trout exposed to three pH levels (7.6, 5.6, and 4.5) during a 20-day exposure, spawning occurred in the pH 7.6 and 5.6 groups, but not in the pH 4.5 group (Roy et al., 1990). Reduced egg production due to lowered pH is also dependent on calcium concentrations. Low environmental calcium concentrations in low pH waters are responsible for lower plasma calcium concentrations in many fish species (Wood et al., 1990). The effect of reduced plasma calcium inhibits the formation of vitellogenin for egg yolk synthesis (Yeo and Mugiya, 1997). Laboratory experiments measuring brook trout response to pH showed that egg-to-larva survival at pH 5.2 was sixty-nine percent of the survival at pH 6.5 (Marschall and Crowder, 1996). Adult reproduction may be affected at pH and aluminum concentrations observed in headwater streams in the GRSM (Fiss and Carline, 1993).

Physical habitat and hydrological characteristics

Like water chemistry, physical habitat and hydrologic characteristics can affect the population structure of freshwater fish. Brook trout are present in streams at high elevations with cold water, steep gradients, small channels, and fast water velocities (Baldigo and Lawrence, 2001). Low gradients and stable flows are common features of rivers with high brown trout and rainbow trout abundance (Jowett, 1990).

The flow regime of a stream affects the structure, composition, and productivity of fish communities by regulating abiotic habitat conditions and biotic processes (Poff et al., 1997; Richter et al., 1996). Floods and droughts are the major forms of natural hydrologic disturbances that regulate riverine biological communities (Cattaneo et al., 2002). The natural flow regime of a river plays a critical role in determining the distribution, diversity, and abundance of riverine species. A river's flow regime can affect abiotic characteristics such as flow depth and velocity, temperature, oxygen content, turbidity, streambed substrate, and morphology (Richter et al., 1996), and biotic processes in the riverine community (Poff et al., 1997). The timing, frequency, duration, and magnitude of a disturbance may dictate the success or failure of populations in stream ecosystems (Lake, 2000). The severity of a hydrologic event to biota is dependent on the timing of the event relative to the fish developmental stage. Mountain headwater streams, such as those in the GRSM, are more sensitive to variations in streamflow which affects the availability of habitat for communities to grow, forage, and reproduce (Elwood and Waters, 1969).

High-elevation stream communities are especially prone to large disturbances caused by floods (Roghair et al., 2002; Swanson et al., 1998). Floods may cause rapid effects on fish populations primarily from high in-stream velocities and debris flow, which may cause death, displacement, or reduce effective habitat (Elwood and Waters, 1969). Harvey (1987) concluded that the ability of fish to maintain their position in-stream under high flows increased rapidly with size because larger fish are stronger and have better swimming abilities. Extreme high flows can destroy or damage fish eggs or larvae in redds (Carline and McCullough, 2003) or increase sedimentation of fine material on spawning gravel decreasing oxygen availability to developing embryos and physically trapping emerging alevins (Jensen and Johnsen, 1999). Additionally, fish can be stranded in isolated pools from the main channel once a flood flow has receded. Severe flooding in salmonid streams commonly destroys the year class of fish that are still incubating or have recently emerged and can take two to three years to recolonize (Carline and McCullough, 2003). Timing is important as winter flooding can destroy redds of fall-spawning brook trout and spring floods can destroy redds of spring-spawning rainbow trout.

Droughts can affect the fish communities by physically decreasing the surface area and volume of the water body, and by altering the water quality, particularly temperature and oxygen (Closs and Lake, 1996; Cowx et al., 1984; Matthews and Marsh-Matthews, 2003). Drought reduces habitat leading to increased fish density, causing increased biotic interactions including predation and competition for diminishing food. Low water levels may prevent aquatic species access to spawning grounds leading to possible loss of a year class (Lake, 2003; Richter et al., 1996). Severe drought may reduce groundwater flow into streams thereby negatively affecting spawning grounds for fish species that utilize discharging groundwater to regulate temperature, chemistry, and hydrology within redds (Curry and Noakes, 1995). Low flow can cause brook trout eggs to become infected with Saprolegnia, thereby reducing embryo survival (Hakala and Hartman, 2004). Lack of food abundance has been attributed to a loss of benthic macroinvertebrate populations, reduction in surface area available to catch falling terrestrial insects, and diminished invertebrate drift, the principal food source for salmonids (Canton et al., 1984; Hakala and Hartman, 2004).

Parker (2008) found the abundance of young-of-the-year (YOY) brook and rainbow trout significantly declined after extreme floods and droughts in 138 watersheds in the GRSM. In particular, low-flows during droughts significantly reduced recruitment for both brook and rainbow trout, which is likely due to decreased spawning habitat (Parker, 2008). Brook trout populations in larger low-elevation streams showed more stability compared to smaller headwater streams. Extreme flood conditions significantly lowered YOY trout abundance, particularly rainbow trout populations (Parker, 2008). Low flow (drought) conditions reduced fish biomass and were highly correlated with lower abundance and biomass of brook trout. These impacts were most pronounced in low elevation streams, which provide less temperature refugia and increased competition pressures from rainbow trout. Brook trout repopulated stream reaches in 2-3 years following low flow regimes (Parker, 2008).

Chapter III: The Influence of Basin Characteristics on Stream Acidification in the Great Smoky Mountains National Park

This chapter is revised based on a paper to be published by Keil J. Neff, John S. Schwartz, Stephen E. Moore, and Matt A. Kulp.

My primary contributions to this paper included (i) developing problem into a work, (ii) identifying study objectives, (iii) selecting sites and installing monitoring equipment, (iv) conducting field experiments and laboratory analyses, (v) analyzing data, (vi) pulling contributions into a single paper, and (vii) primarily authoring the paper.

Neff, K. J., Schwartz, J. S., Moore, S. E., Kulp, M. A., (2010). "The Influence of Basin Characteristics on Stream Acidification in the Great Smoky Mountains National Park, USA." Journal of the American Water Resources Association, *in preparation for submittal*.

Abstract

Relationships between stream chemistry and elevation, area, Anakeesta geology, soil properties, and dominant vegetation were evaluated to identify the influence of basin characteristics on acidification response in eight streams of the Great Smoky Mountains National Park. Statistical analyses were employed to determine differences between baseflow and stormflow chemistry, and relate basin-scale factors governing local chemical processes to stream chemistry. Following precipitation events, stream pH was reduced and aluminum concentrations increased, while the response of ANC, nitrate, sulfate, and base cations varied. Decreasing concentrations of sodium (-4.72 µeq/L) and silicon (-0.55 ppm), and increasing concentrations of magnesium (+2.06 µeq/L), calcium (+8.27 µeq/L), potassium (+4.15 µeq/L), and DOC (+1.83 µeq/L) were observed from baseflow to stormflow. Streams at higher elevations (>975 m) had significantly lower pH, ANC, sodium, and silicon and higher nitrate concentrations ($p<0.05$). Smaller streams (< 10 km^2) had significantly lower nitrate, sodium, magnesium, silicon, and base cation concentrations ($p<0.05$). In stormflow, streams in basins with Anakeesta geology (>10%) had significantly lower pH and sodium concentrations, and higher aluminum concentrations. Weight-averaged soil parameters and percentage of forest types in basins additionally contributed to unique stream acidification response. Several basin characteristics were highly correlated demonstrating the interrelatedness of topographic, geologic, soil, and vegetative parameters; these included elevation, drainage area, basin slope, chemical and physical soil characteristics, and percentage of forest types. These interrelated factors influenced baseflow and stormflow chemistry in these streams.

1. Introduction

Atmospheric acid deposition has impacted baseflow and stormflow water chemistry in some streams of the Great Smoky Mountains National Park (GRSM), but not all streams are affected to the same extent (Cai et al., 2009; Deyton et al., 2009; Flum and Nodvin, 1995; Robinson et al., 2008). During stormflow, pH and ANC are depressed in some GRSM streams, yet the variability of nitrate, sulfate, cation dilution, and organic acids contributing to pH and ANC depressions exemplifies the complexity of watershed process that affect episodic acidification (Cai, 2010; Deyton et al., 2009). Although the influence of basin factors on baseflow stream chemistry and watershed processes is recognized (Andersson and Nyberg, 2009; Clow and Sueker, 2000; Sullivan et al., 2007), the function of physical basin characteristics on the episodic acidification response during stormflow is not fully understood. To effectively manage and protect aquatic resources in GRSM streams, it is necessary to understand how physical basin characteristics influence stream chemistry, especially those causing episodic acidification.

Two known sources of acid in GRSM surface waters include atmospheric acid deposition and disturbed Anakeesta phyllite (Deyton et al., 2009; Huckabee et al., 1975). The GRSM receives elevated rates of acid deposition in the form of sulfur and nitrogen compounds (Baumgardner et al., 2003; NADP, 2009; Weathers et al., 2006), which contribute to the acidification of poorly buffered surface waters (Deyton et al., 2009). Acid deposition has decreased during the past quarter century (NADP 2009); however, there has been little recovery of acidified stream waters and soils in the GRSM (Robinson et al., 2008) or other acid sensitive areas in the eastern U.S.A. (Lawrence et al., 2008; Webb et al., 2004). A potentially significant source of acidification of streams in the GRSM is Anakeesta, a carbonaceous and sulfidic slate, which when disturbed and exposed to air and water oxidizes to release acid, aluminum and other heavy metals to adjacent waterbodies (Huckabee et al., 1975; Kucken et al., 1994). Anakeesta geology, comprising less than 8% of the GRSM, is primarily found in high elevation basins. During the 1970’s, aquatic biota were extirpated from Alum Cave Creek and Walker Camp Prong due to natural disturbances of Anakeesta (Bacon and Maas, 1979).

Hydrochemical process-oriented research has added significantly to the understanding of the factors that control water chemistry (Cai et al., 2010; Tranter et al., 1994; Wellington and Driscoll, 2004; Wigington et al., 1996b). These small-scale studies are dominant in the literature because of the large spatial and temporal variability of factors that influence stream chemistry (Likens and Buso, 2006). Variations in stream water chemistry can result from differences in physical and chemical properties of soil which influence the rates and types of geochemical and biological reactions (Billett and Cresser, 1992; Cai et al., 2009). Stormflow water is more acidic than baseflow because the majority of the source water is routed rapidly through the upper layers of the soil, which supply protons from acid deposition and organic processes for transport (Cai et al., 2009; Wigington et al., 1996b). Long-term acid deposition may cause a loss of exchangeable base cations resulting in base cation dilution during stormflows (Fernandez et al., 2003). Additionally, organic acids and nitrate contribute to stream acidification, yet are dependent on vegetation and season, and governed by biogeochemical processes including forest uptake, nitrification and mineralization, and soil saturation (Andersson and Nyberg, 2009; Dittman et al., 2007; Mulholland, 2004; Wright et al., 2006).

Studies that have addressed larger-scale environmental problems have associated system response with topographical, geological, pedological, vegetative, and climatological factors (Sullivan et al., 2007; van Dobben and de Vries, 2010; Wolock et al., 1989). Topography controls hydrological factors that influence the chemical composition of surface waters by affecting soil water content, flowpaths and residence times (McGuire et al., 2005). High elevation headwater streams in the Appalachians are susceptible to acidification because of higher rates of acid deposition, precipitation and base cation leaching, and steeper slopes in less well-developed soils that limit buffering capacity (Deviney et al., 2006; Weathers et al., 2006). Increases in proton, aluminum, and DOC concentrations were reported in streams with higher elevations and smaller drainage areas in the Hubbard Brook Valley (Likens and Buso, 2006). Clow and Seuker (2000) observed acidity, alkalinity, base cations and nitrate stream concentrations were related to slope, vegetation, and distribution and age of surficial materials in basins of the Rocky Mountain National Park. At a regional scale, Sullivan et al. (2007) modeled the presence/absence of acid sensitive streams using lithology, basin area, soils type, and forest type basin variables.

Stream acidification can have damaging effects on the health of aquatic ecosystems and fish populations (Baldigo and Lawrence, 2001); and is suspected to have contributed to the extirpation of native brook trout (*Salvelinus fontinalis*) in some GRSM headwater streams (Neff et al., 2009). Although the majority of water chemistry data collected in the GRSM is baseflow, episodic acidification is of particular concern as fish can die or experience sublethal physiological stress resulting in displacement, less successful reproduction, impaired swimming, and decreased growth (Baldigo et al., 2007; MacAvoy and Bulger, 2004; Neff et al., 2009). The chemical response to the same rain event varies in different streams in the GRSM; in streams with higher proton and aluminum concentrations during stormflow, brook trout experience acute sublethal physiological distress (Neff et al., 2009).

This research focuses on understanding how basin characteristics in the GRSM influence stream water chemistry, especially those governing the acidification of surface waters. The objectives of this research were to 1) develop relationships between baseflow and stormflow chemical constituents, and 2) examine the effects of elevation, basin area, and Anakeesta geology on chemical response to episodic stream acidification. Based on a preliminary analysis, a secondary objective was constructed to investigate the influence of physical and chemical soil properties and dominant vegetation on stream chemistry. Using available spatial data, baseflow and stormflow water chemistry were characterized in distinct watersheds of the GRSM and linear regression models were developed to predict ionic species concentrations in stormflow from basin characteristics and baseflow chemistries.

2. Methods

2.1 Study area

The GRSM, with an area of 2,108 km^2, is located in the Blue Ridge physiographic region of the southern Appalachian in eastern Tennessee and western North Carolina (Figure 7). This physiographic region is characterized by rugged topography, heavily forested slopes, and steep mountain streams which flow into the Tennessee River system. Altitudes in the GRSM range from 300 m to 2,025 m. GRSM watersheds are characterized by steep gradients and thin sandy loams that provide poor buffering capacities. Streambeds are dominated by boulder and cobble, and gradients of the stream channels increase with elevation (Larson and Moore, 1985). The climate of GRSM is perhumid mesothermal with seasonal temperature variation and precipitation distributed throughout the year (Busing, 2005). The average annual rainfall varies significantly throughout the

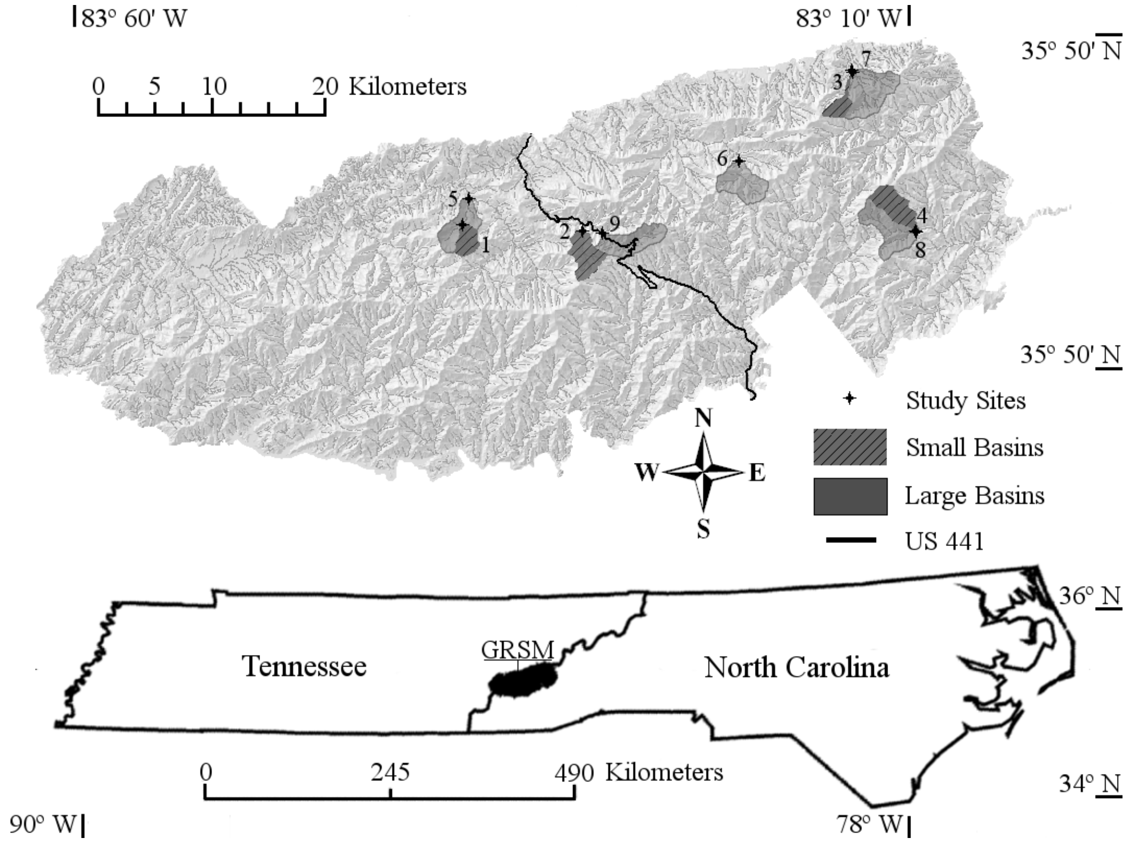

Figure 7: Location of water quality monitoring sites in the Great Smoky Mountains National Park. Site numbers correspond with block unit illustrated in Table 1: Newt Prong (1); Road Prong (2); Rock Prong (3); Lost Bottom Creek (4); Jakes Creek (5); Eagle Rocks Prong (6); Cosby Creek (7); Palmer Creek (8); and Walker Camp Prong (chemically impacted from limestone/dolomite aggregates applied to US 441), (9).

park with lower elevations generally receiving near 127 cm and some higher elevation sites near 216cm (Busing 2005). The mean (standard deviation) annual precipitation at the Tremont Institute (420 m elevation) is 155 (25) cm/year (NADP, 2009). During the period 2006 through 2007, the average annual precipitation was 139 cm; from 2008 through 2009, the average annual precipitation was 161 cm.

2.2 Study design

Eight stream study sites were selected in GRSM basins considering a symmetric block design (considering basin area, site elevation, and presence of Anakeesta geology), with one site per block unit to allow greater precision in the estimation of the source of variation under study (Figure 7). Basin area was defined as large (10–20 km^2) or small (1–10 km^2); site elevation was defined as high (> 975 m) or low (< 975 m); Anakeesta occurrence was defined as present (>10%) or absent (0%). These criteria were selected because of the variation in acidification response in streams with different basin characteristics previously reported (Deyton et al., 2009). The eight test sites (Newt Prong, Road Prong, Rock Creek, Lost Bottom Creek, Jakes Creek, Walker Camp Prong, Cosby Creek and Palmer Creek) were studied from May 2008 through September 2009.

The original design included Walker Camp Prong, but it was removed from block design analyses because it was discovered during the study period that the water chemistry at this site was anthropogenically impacted by the application of a limestone/dolomite aggregate for winter road traction. The concentrations of calcium, magnesium, and ANC in Walker Camp were significantly higher than the other seven study sites in baseflow and stormflow. During the 2009/2010 winter, about 600 tons of the limestone/dolomite aggregate was applied to highway US 441, which traverses 7.3 km through this basin and within 50 m of the water quality monitoring site. In a concurrent study, Grell (2010) found soil calcium concentrations in this basin were also significantly higher than those found in the other seven study basins. To fill this block unit, Eagle Rocks Prong, with the same physical basin characteristics criteria (block unit) as Walker Camp Prong (investigated from April 2006 through December 2007; Deyton et al., 2009), was used for block design analyses. We believe the effects on water chemistry from the temporal variability in climate and hydrology was minimal in contrast to the effects of the road application of the limestone/dolomite aggregate in the Walker Camp basin.

2.3 Water chemistry collection and analysis

Water samples were collected during baseflow and stormflow stream conditions, and continuous water quality data logging was conducted at the study sites. Baseflow grab samples were obtained monthly and more frequently before storm events. Stormflow water samples were collected utilizing an automated water sampler (ISCO® 6712) at Eagle Rocks and passive water samplers (on the rising limbs of the hydrographs; Deyton et al., 2009) at the other study sites. Conductivity, pH, temperature, and stage height were monitored at the Eagle Rocks site using a 6920 YSI® data sonde, in which parameters were measured at 15 minute intervals. These parameters were measured every 30 minutes at the other study sites using Eureka Manta1® data sondes.

At the University of Tennessee, water samples were analyzed for the following parameters: conductivity, pH, ANC (Mantech™ PC-Titration Plus); SO_4^{2-}, NO_3^{2-}, Cl^-, NH_4^+, (Dionex™ IC); Al, Ca, Cu, Fe, K, Mg, Mn, Na, Si, and Zn (Thermo-Electron™ Intrepid II ICP-AES, vacuum-filtered (0.45 μm) and acidified. Cation sums were calculated for samples by adding the equivalent concentrations of NH_4^+, Na, Ca, Mg, and K. Likewise, anion sums were calculated by adding equivalent concentrations of SO_4^{2-}, NO_3^{2-}, and Cl^-.

Approximately 25% of all samples were analyzed for dissolved organic carbon (Shimadzu TOC analyzer, vacuum-filtered (0.45 μm)).

2.4 Characterizing topographical, geological, soil, and vegetative parameters in study basins

ArcGIS® 9.2 (Environmental Systems Research Institute (ESRI)) was utilized to determine basin characteristics using a 10-meter Digital Elevation Model (USGS), and digital geological (Southworth et al., 2004), soil (USDA-NRCS, 2009), and vegetation (Madden et al., 2004) maps. ArcHydro tools (Center for Research in Water Resources) and Spatial Analyst tools (ESRI) were used to delineate basins and compute basin areas, elevations, and slopes; the Spatial Analyst Zonal Statistics Tool (ESRI) enabled detailed geological, soil and vegetation information to be computed for each basin.

General soil characteristics, including soil reaction (soil pH), effective cation exchange capacity (CEC), organic percentage, saturated hydraulic conductivity (Ksat), average soil depths, and average soil slopes, were applied to each basin. This was accomplished by calculating weighted averages (with respect to soil components within soil types, and respective horizons within soil components) for each soil parameter and computing an area weighted average of these parameters for each basin.

Vegetative characteristics were summarized in study basins by calculating the percent area of the dominant over-story vegetation forest types in each basin. The primary forest types in the eight basins included sub-alpine mesic forests and woodlands (SAMF), high elevation mesic to submesic forests (HEMF), low and mid elevation mesic to submesic forests (LEMF), low and mid elevation subxeric to xeric forests and woodlands (LEXF), and shrublands or shrub understory (Shrub). SAMF forests are largely comprised of Spruce and Fir and can be intermixed with Northern Hardwoods. Southern Appalachian Hardwoods, along with Yellow Birch, Eastern Hemlock, and Northern Red Oak primarily constitute HEMF forests. LEMF forests consist of Southern Appalachian Cove Hardwoods, Oak and mixed Hardwoods, Eastern Hemlock, Eastern White Pine, and Southern Appalachian Early Successional Hardwoods. LEXF are comprised of Chestnut Oak, Red Maple, mixed Hardwoods, and Eastern White Pine. Jackson et al. (2004) provides a complete description of these forest types.

Pearson pairwise correlation analyses were performed in order to investigate the relationships between mean basin elevation, mean basin slope, basin area, percent area with Anakeesta geology, and vegetative and soil characteristics in the eight study basins. Significant differences were reported when $p < 0.05$.

2.5 Comparison of water chemistries between baseflow and stormflow

Means and standard deviations of measured chemical constituents in baseflow and stormflow samples were calculated for the eight study sites. Additionally, chemical averages were calculated for Walker Camp Prong to quantify the impact of road-applied limestone/dolomite aggregate on stream water quality. To represent the stormflow chemistry for each storm event at each site, the sample with the minimum ANC concentration from that event (typically corresponding with maximum conductivity and minimum pH) was selected.

Analysis of variance (ANOVA) Tukey-Kramer honestly significant difference (HSD) tests were used to determine differences between baseflow and stormflow chemistry in a) block basins and b) block unit classes (i.e. high and low elevation, small and large area, and present and absent Anakeesta). Significant differences were reported when $p < 0.05$. Linear regression was performed on baseflow pH versus stormflow pH, and baseflow ANC versus stormflow ANC to elucidate the baseflow/stormflow relation. The JMP platform (SAS Institute Inc.) was used for statistical analyses.

2.6 Relations between physical basin characteristics and water chemistry

Tukey-Kramer HSD tests were used to determine differences ($p < 0.05$) in chemical constituents among block basins and block unit classes for baseflow and stormflow. Principal components analysis (PCA) was used to examine the relations between block basins and examine the loadings of the chemical variables in baseflow and stormflow stream water on block basins. Matrices for the PCA analyses comprised of the average baseflow and stormflow constituents (pH, ANC, SO_4, NO_3, Cl, NH_4, Al, Ca, K, Mg, Na, Si, DOC) in each study basin. Principal components were considered for evaluation when more than 80% of the variance was explained by the model, and individual component eigenvalues were greater than one. Additionally, Spearman bivariate correlation analyses were performed in order to investigate the relationships between physical basin variables and water chemistry variables in baseflow and stormflow. Correlation basin variables included the three block-designed criteria (basin area, Anakeesta percent, and site elevation), surrogate variables Anakeesta area, mean basin elevation, and mean basin slope, and soil and vegetative characteristics. The basin parameters that correlated most strongly with the chemistry variables were reported ($p < 0.01$). To relate chemical constituent concentrations in baseflow and stormflow to soil and vegetation basin characteristics, canonical correspondence analysis (CCA) was utilized. The primary matrices were the same as used in the PCA analyses excluding ANC concentrations. Secondary matrices were comprised of the soil and vegetative characteristics for each basin. The JMP platform was used for ANOVA and Spearman correlation analyses; PC-ORD was used for PCA and CCA tests.

2.7 Stepwise multiple regressions

Using stepwise multiple regression, models were developed in JMP to predict stormflow pH and ANC concentrations from baseflow chemical constituents independently and in conjunction with basin characteristics. Only significant models ($p<0.05$) with associated independent variables and intercepts ($p<0.05$) were considered. Multicollinearity was addressed using the variance of inflation factor (VIF) and informal multicollinearity diagnostics including Spearman bivariate correlations. Independent variables with VIF > 10 were removed in reverse order of the explanatory ability to produce the best models while minimizing multicollinearity. Cook's D statistic was used to evaluate individual observations with high leverage on regression models. The simplest model that explained a comparable amount of variability was chosen as the final model.

3. Results

3.1 Characterizing topographical, geological, vegetative, and soil parameters in study basins

As designed, study basins had distinct topographic and geologic attributes with respect to elevation, basin area, and Anakeesta geology (Table 1). Large and small basins had average areas of 13.3 km^2 and 6.3 km^2 respectively. Basins with Anakeesta had an average of 28% Anakeesta in their particular basin areas. High and low elevation sites had average elevations of 1020 m and 690 m. In high and low elevation basins the mean slopes were 25.5º and 28.2º; slopes in Anakeesta and area blocks were similar.

Study basins demonstrated distinct area-weighted average soil characteristics (Table 2). The average soil reaction rate (pH) was 4.46 pH units for all basins. Road Prong and Eagle Rocks basins had the lowest average soil pH; within their respective basins, 87% and 75% of the average soil pH was less than 4.5. The high elevation, no

Table 1: Topographic and geologic basin characteristics of study sites.

Basin	Block	Elevation (m)	Area (km^2)	Anakeesta (km^2)	Mean Basin Elevation (m)	Mean Basin Slope
Newt	1	Low (870)	Small (4.09)	Present (1.97)	1214	23.6º
Road	2	High (1090)	Small (8.60)	Present (1.75)	1529	30.0º
Rock	3	Low (630)	Small (3.63)	Absent (0)	1249	25.8º
Lost Bottom	4	High (1015)	Small (8.45)	Absent (0)	1423	26.4º
Jakes	5	Low (660)	Large (12.01)	Present (4.02)	1096	22.5º
Eagle Rocks	6	High (975)	Large (10.47)	Present (1.17)	1445	30.5º
Cosby	7	Low (610)	Large (17.51)	Absent (0)	1112	28.2º
Palmer	8	High (990)	Large (19.99)	Absent (0)	1380	25.8º
Mean(SD)		855(194)	10.59(5.82)	1.11(1.44)	1306(161)	26.6(2.84)º

Table 2: Weighted averages (per area) of physical and chemical soil properties in study basins. Soil data from USDA-NRCS, 2009.

Basin	Reaction Rate (pH)	Effective CEC (meq/100g)	Organic Matter (%)	Ksat (cm/hr)	Subsoil Depth (cm)	Slope (%)
Newt	4.47	4.51	5.26	3.64	73.05	56.11
Road	4.17	5.72	5.64	4.91	72.07	56.99
Rock	4.39	5.00	6.43	4.30	80.09	56.92
Lost Bottom	4.60	5.33	6.91	3.10	112.10	56.84
Jakes	4.58	4.32	6.07	3.10	84.53	49.83
Eagle Rocks	4.33	4.13	8.59	4.78	80.42	60.71
Cosby	4.51	4.76	6.45	3.37	85.64	52.94
Palmer	4.61	5.17	6.73	2.66	117.97	56.88
Mean(SD)	4.46(0.15)	4.87(0.54)	6.51(1.00)	3.73(0.84)	88.23(17.29)	55.90(3.23)

Anakeesta basins (Palmer and Lost Bottom) had the highest average soil pH, and the deepest average subsoil depths (112 cm and 118 cm respectively). The average soil depth in all the study basins was 88 cm. Eagle Rocks and Road Prongs were found to have the highest average soil slopes and saturated hydraulic conductivities of the study basins. The average percentage of soil organic matter in the study basins was 6.5%. In Eagle Rocks, the basin with the highest percentage of organic matter, the average was 8.6%.

The vegetation compositions among study basins were also diverse (Table 3). The high elevation, no Anakeesta basins (Palmer and Lost Bottom) included all forest types and had highest percentage of high elevation mesic to sub-mesic forests. Road and Eagle Rocks basins (high elevation, Anakeesta) primarily consisted of sub-alpine and high elevation forests (99% and 89% of respective basin areas). Low and mid elevation mesic to submesic forests and shrublands were the primary vegetation forest types in low elevation, Anakeesta basins (79% and 88% of Newt and Jakes basin areas). Cosby and Rock basins were primarily comprised of high, low and mid elevation mesic to submesic forests.

Table 3: Dominant vegetation forest types in study basins (percent area). Explanation of abbreviated forest types: SAMF – sub-alpine mesic forests and woodlands, HEMF – high elevation mesic to submesic forests, LEMF – low and mid elevation mesic to submesic forests, LEXF – low and mid elevation subxeric to xeric forests and woodlands, and Shrub – shrublands or shrub understory. Data from Madden et al., 2004.

Basin	SAMF	HEMF	LEMF	LEXF	Shrub
Newt	0.0%	18.6%	49.2%	1.8%	29.7%
Road	66.0%	32.9%	0.0%	0.0%	1.0%
Rock	18.1%	36.7%	35.4%	0.2%	9.5%
Lost Bottom	15.7%	41.9%	20.2%	10.8%	9.0%
Jakes	0.0%	9.7%	55.3%	9.6%	22.3%
Eagle Rocks	50.8%	37.8%	4.4%	0.0%	5.2%
Cosby	5.4%	26.5%	55.6%	5.0%	5.4%
Palmer	8.8%	42.7%	34.1%	6.6%	6.2%
Mean(SD)	21.6(24.5)%	30.9(11.7)%	31.8(21.8)%	4.3(4.4)%	11.0(9.8)%

Some basin characteristics were highly correlated (Table 4), demonstrating the interrelatedness of basin, soil, and vegetative variables. Mean basin elevation and mean basin slope were positively correlated with average soil slope, sub-alpine mesic forests, and high elevation mesic forests; and negatively correlated with low and mid mesic elevation forests. The percentage of sub-alpine forests in basins was negatively correlated with low and mid elevation mesic forests and soil pH, and positively correlated with average soil saturated hydraulic conductivity. Average soil pH was positively correlated with average soil depth and low elevation xeric forests; these parameters were negatively correlated with saturated hydraulic conductivity respectively.

3.2 Comparison of water chemistries between baseflow and stormflow

Following precipitation events, the stream water pH was significantly reduced ($p<0.05$) and aluminum concentrations increased in seven of the eight study sites, while the

Table 4: Pearson correlation coefficients for physical basin characteristics.

	Soil Properties				Vegetation Classes					Topography and Geology		
	Soil pH	Ksat (cm/hr)	Soil Depth (cm)	Soil Slope	SAMF	HEMF	LEMF	SEXF	Shrub	Basin Area (km2)	Basin Elevation (m)	Anakeesta %
Soil pH												
Ksat	-0.96*											
Soil Depth	0.72§	-0.73§										
Soil Slope	-0.46	0.54	0.04									
SAMF	-0.86*	0.83*	-0.33	0.62								
HEMF	-0.11	0.14	0.53	0.76§	0.42							
LEMF	0.65	-0.66	0.03	-0.77§	-0.92*	-0.64						
SEXF	0.84*	-0.85*	0.70	-0.56	-0.58	-0.14	0.40					
Shrub	0.39	-0.33	-0.24	-0.43	-0.65	-0.73§	0.63	0.20				
Area	0.42	-0.53	0.56	-0.25	-0.18	0.13	0.19	0.41	-0.39			
Basin Elev	-0.49	0.46	0.18	0.79§	0.80§	0.73§	-0.95*	-0.29	-0.59	-0.10		
Anak %	-0.12	0.09	-0.57	-0.28	-0.13	-0.78§	0.24	-0.13	0.80§	-0.41	-0.26	
Mean Slope	-0.67	0.66	-0.15	0.63	0.83*	0.57	-0.75§	-0.51	-0.83*	0.14	0.65	-0.46

Numbers are *r values.*
* $p \leq 0.01$.
§ $p \leq 0.05$.

response of ANC, nitrate, sulfate, and base cations varied (Table 4). Rock Creek was the only site that did not have a significant decrease in pH or increase in aluminum concentration. Six of the eight block-designed study sites did not have a significant difference in ANC concentrations between baseflow and stormflow. Only Eagle Rocks Prong (high elevation, large drainage area, Anakeesta) had a significant decrease in ANC concentration. Jakes Creek (low elevation, large drainage area, Anakeesta) demonstrated asignificant increase in ANC from baseflow to stormflow. Only Road Prong (high elevation, small drainage area, Anakeesta) demonstrated anion dilution with significant decreases in concentrations of chloride (-1.87 μeq/L) and nitrate (-8.45 μeq/L) from baseflow to stormflow. Eagle Rocks Prong, with the highest average sulfate concentrations of the sites in baseflow (50.90 μeq/L) and stormflow (64.73 μeq/L), was the only site that had a significant increase in sulfate concentration. The other seven sites had average sulfate concentrations of 36.5 μeq/L in baseflow and 38.74 μeq/L in stormflow. Jakes Creeks had a significant increase in base cations of 38.1 μeq/L from baseflow to stormflow, whereas the average increase was 10.47 μeq/L. The base cation concentrations in Road and Eagle Rocks Prong decreased (-0.32 μeq/L and -2.59 μeq/L respectively).

The general trend from baseflow to stormflow chemistry was decreased sodium (-4.72 μeq/L) and silicon (-0.55 ppm) concentrations, and increased magnesium (+2.06 μeq/L), calcium (+8.27 μeq/L), potassium (+4.15 μeq/L), and DOC (+1.83 μeq/L) concentrations (Table 4). Five of the sites, excluding Road and Eagle Rocks Prongs and Rock Creek had significant increases in magnesium, calcium or both. All sites had higher potassium concentrations in stormflow (13.81 ppm) than in baseflow (9.66 ppm); these increases were significant at Road Prong, and Jakes and Cosby Creeks. Significant increases ($p<0.05$) in DOC concentrations were

Table 5: Means and standard deviations of selected chemical constituents for block water quality monitoring sites and Walker Camp Prong (excluded because of contamination from limestone/dolomite aggregates applied for road traction). ANOVA Tukey's HSD multiple comparison technique applied between sites (different letters indicate significant difference (p<0.05)) and between baseflow and stormflow (underlines indicate significantly lower and overlines indicate significantly higher (p<0.05)).

		Block 1[1]	**Block 2**[2]	**Block 3**[3]	**Block 4**[4]	**Block 5**[5]	**Block 6**[6]	**Block 7**[7]	**Block 8**[8]	**Unique**
	Basin	Newt	Road	Rock	Lost Bottom	Jakes	Eagle Rocks	Cosby	Palmer	Walker Camp
Baseflow Water Chemistry	**N (N DOC)**	19(4)	18(4)	17(3)	15(3)	18(4)	26(2)	16(2)	17(3)	17(3)
	pH	6.50(0.13)B	6.30(0.14)C	5.96(0.11)D	6.56(0.12)AB	6.67(0.09)AB	5.39(0.35)E	6.59(0.13)AB	6.64(0.12)AB	6.74(0.19)A
	ANC(μeq/L)	40.6(10.1)BC	27.5(11.8)CD	11.1(5.0)DE	46.4(10.0)BC	57.5(10.1)B	-0.3(4.2)E	53.0(21.5)B	56.6(13.6)B	91.7(57.2)A
	N0$_3$(μeq/L)	5.4(3.4)D	31.4(4.8)B	26.7(7.0)BC	7.9(3.5)D	4.7(2.3)D	49.6(6.8)A	24.0(4.0)C	8.7(3.8)D	27.8(7.0)BC
	SO$_4$(μeq/L)	31.9(1.6)C	47.8(3.2)B	47.2(4.1)B	23.2(2.8)DE	26.5(2.2)CD	50.9(9.3)B	44.8(4.7)B	18.8(2.1)E	88.7(16.9)A
	Cl(μeq/L)	11.7(1.3)B	12.2(0.8)B	12.5(2.2)B	11.1(1.2)B	11.0(1.3)B	11.9 (2.0)B	12.0 (1.2)B	11.1(1.4)B	22.2(6.1)A
	Al (ppm)	0.03(0.02)B	0.05(0.03)AB	0.06(0.04)AB	0.03(0.02)B	0.02(0.02)B	0.07(0.05)A	0.03(0.02)B	0.03(0.02)B	0.03(0.03)B
	Si(ppm)	2.6(0.4)B	2.1(0.2)C	2.1(0.2)C	2.8(0.4)AB	2.9(0.3)AB	2.0(0.2)CD	2.7(0.4)AB	3.0(0.4)A	1.7(0.1)D
	Na(ppm)	33.1(4.6)BC	26.4(2.3)D	27.5(2.7)D	35.6(4.8)ABC	35.0(3.5)ABC	27.3(3.8)D	38.8(6.1)A	37.1(5.1)AB	32.3(4.3)C
	Mg(ppm)	16.4(0.6)E	26.6(2.1)C	22.2(1.9)D	16.7(1.8)E	17.2(1.5)E	31.5(2.7)B	29.4(2.1)BC	17.8(1.6)E	56.1(8.7)A
	Ca(ppm)	44.5(6.3)DE	64.8(4.9)B	48.2(6.2)DE	39.3(8.2)E	48.5(7.9)DE	53.5(4.1)CD	62.6(5.2)BC	41.9(8.3)E	146.8(24.1)A
	Σcations(μeq/L)	104.6(10.1)E	126.7(8.2)BC	106.2(9.0)DE	103.2(13.7)E	111.1(11.9)CDE	120.9(8.3)CD	141.2 (11.7)B	108.6(14.9)DE	240.4(8.5)A
	DOC(mg/L)	0.7(0.2)B	0.9(0.1)B	1.0(0.3)B	0.9(0.2)B	1.0(0.5)B	2.6(0.2)A	0.7(0.2)B	1.0(0.1)B	0.8(0.2)B

[1] Small (1–10 km^2); low elevation (< 975 m); Anakeesta present (>10%).
[2] Small (1–10 km^2); high elevation (> 975 m); Anakeesta present (>10%).
[3] Small (1–10 km^2); low elevation (< 975 m); no Anakeesta present (0%).
[4] Small (1–10 km^2); high elevation (> 975 m); no Anakeesta present (0%).
[5] Large (10–20 km^2); low elevation (< 975 m); Anakeesta present (>10%).
[6] Large (10–20 km^2); high elevation (> 975 m); Anakeesta present (>10%).
[7] Large (10–20 km^2); low elevation (< 975 m); no Anakeesta present (0%).
[8] Large (10–20 km^2); high elevation (> 975 m); no Anakeesta present (0%).

Table 5 (continued): Means and standard deviations of selected chemical constituents for block water quality monitoring sites and Walker Camp Prong (excluded because of contamination from limestone/dolomite aggregates applied for road traction). ANOVA Tukey's HSD multiple comparison technique applied between sites (different letters indicate significant difference (p<0.05)) and between baseflow and stormflow (underlines indicate significantly lower and overlines indicate significantly higher (p<0.05)).

		Block 1[1]	**Block 2[2]**	**Block 3[3]**	**Block 4[4]**	**Block 5[5]**	**Block 6[6]**	**Block 7[7]**	**Block 8[8]**	**Unique**
	Basin	Newt	Road	Rock	Lost Bottom	Jakes	Eagle Rocks	Cosby	Palmer	Walker Camp
Stormflow Water Chemistry	**N (N DOC)**	12 (3)	16 (5)	13 (4)	12 (4)	12 (4)	8 (3)	11 (5)	8 (3)	16(5)
	pH	$\underline{\text{6.26(0.11)BC}}$	$\underline{\text{5.79(0.46)D}}$	5.93(0.13)CD	$\underline{\text{6.32(0.17)B}}$	$\underline{\text{6.52(0.27)B}}$	$\underline{\text{4.53(0.16)E}}$	$\underline{\text{6.35(0.23)B}}$	$\underline{\text{6.29(0.24)BC}}$	6.85(0.28)A
	ANC(μeq/L)	41.5(14.1)BC	18.5(20.0)CD	13.9(6.1)CD	45.1(20.8)BC	$\overline{\text{77.6(35.5)B}}$	$\underline{\text{-15.4(6.0)D}}$	48.5(29.14)BC	54.7(19.5)BC	$\overline{\text{146.0(66.7)A}}$
	$N0_3$(μeq/L)	4.41(3.1)C	$\underline{\text{23.1(3.7)B}}$	21.3(10.9)B	8.8(6.5)C	7.9(8.6)C	52.2(10.1)A	22.7(10.9)B	8.9(2.3)C	23.8(5.8)B
	SO_4(μeq/L)	30.8(4.4)CD	46.6(5.3)B	44.2(9.5)B	27.7(17.5)D	28.0(9.6)D	$\overline{\text{64.7(10.6)A}}$	46.7(5.9)B	21.2(5.0)D	78.5(20.7)A
	Cl(μeq/L)	10.4(2.7)B	$\underline{\text{10.4(2.4)B}}$	11.6(2.2)B	11.1(2.4)B	11.0(1.9)B	12.4(3.2)B	11.6(2.2)B	10.4(2.0)B	18.3(4.6)A
	Al(ppm)	$\overline{\text{0.08(0.06)C}}$	$\overline{\text{0.19(0.11)B}}$	0.07(0.04)C	$\overline{\text{0.06(0.03)C}}$	$\overline{\text{0.08(0.08)C}}$	$\overline{\text{0.29(0.13)A}}$	$\overline{\text{0.07(0.04)C}}$	$\overline{\text{0.06(0.03)C}}$	$\overline{\text{0.09(0.05)C}}$
	Si(ppm)	$\underline{\text{2.1(0.4)AB}}$	$\underline{\text{1.5(0.3)CD}}$	$\underline{\text{1.9(0.2)BC}}$	$\underline{\text{2.0(0.6)B}}$	$\underline{\text{2.5(0.5)A}}$	$\underline{\text{1.5(0.2)CD}}$	$\underline{\text{2.1(0.4)AB}}$	$\underline{\text{2.2(0.5)AB}}$	$\underline{\text{1.3(0.2)D}}$
	Na(ppm)	$\underline{\text{26.8(3.7)A}}$	$\underline{\text{19.51(2.2)B}}$	26.8(1.8)AB	$\underline{\text{29.5(3.8)A}}$	31.1(3.5)A	$\underline{\text{18.0(1.7)B}}$	$\underline{\text{30.0(2.4)A}}$	$\underline{\text{29.4(4.6)A}}$	$\underline{\text{27.9(7.1)A}}$
	Mg(ppm)	$\overline{\text{18.4(2.7)E}}$	25.3(2.9)CD	22.6(2.3) DE	$\overline{\text{19.3(4.3)DE}}$	$\overline{\text{23.1(5.1)CDE}}$	34.0(4.8)B	30.2(2.5)BC	$\overline{\text{21.3(2.1)DE}}$	$\overline{\text{64.9(11.1)A}}$
	Ca(ppm)	49.8(9.6)BC	67.7(7.4)BC	52.1(8.9)BC	$\overline{\text{47.5(9.0)C}}$	$\overline{\text{75.9(26.9)B}}$	54.4(2.9)BC	$\overline{\text{71.0(10.4)BC}}$	$\overline{\text{53.0(6.4)BC}}$	$\overline{\text{204.7(45.8)A}}$
	Σcations(μeq/L)	110.1(15.2)C	126.4(10.8)BC	116.3(14.6)BC	115.2(14.6)BC	$\overline{\text{149.2(32.2)B}}$	118.3(16.2)BC	150.1(17.3)B	120.5(15.0)BC	$\overline{\text{305.3(59.9)A}}$
	DOC(mg/L)	$\overline{\text{2.7(1.2)A}}$	$\overline{\text{4.0(1.6)A}}$	1.9(1.3) A	2.5(1.6)A	2.7(1.5)A	$\overline{\text{3.4(0.3)A}}$	2.4(1.6)A	$\overline{\text{3.7(0.8)A}}$	$\overline{\text{2.2(1.0)A}}$

[1] Small (1–10 km^2); low elevation (< 975 m); Anakeesta present (>10%).
[2] Small (1–10 km^2); high elevation (> 975 m); Anakeesta present (>10%).
[3] Small (1–10 km^2); low elevation (< 975 m); no Anakeesta present (0%).
[4] Small (1–10 km^2); high elevation (> 975 m); no Anakeesta present (0%).
[5] Large (10–20 km^2); low elevation (< 975 m); Anakeesta present (>10%).
[6] Large (10–20 km^2); high elevation (> 975 m); Anakeesta present (>10%).
[7] Large (10–20 km^2); low elevation (< 975 m); no Anakeesta present (0%).
[8] Large (10–20 km^2); high elevation (> 975 m); no Anakeesta present (0%).

observed in three of the four high elevation sites (Road, Eagle Rocks, and Palmer) and in Newt Prong (low elevation, small drainage area, Anakeesta).

Testing differences in baseflow and stormflow chemistry between block unit classes (i.e. high and low elevation, small and large area, and present and absent Anakeesta) further illuminated the baseflow stormflow relationship. All unit classes, with the exception of large area basins had significantly lower pH in stormflow than baseflow ($p<0.05$). In large basins, pH decreased 0.19 units on average, whereas in the other five block unit classes, the average decrease was 0.26 units. Among unit classes, there were no significant differences in ANC or anion concentrations. Significant decreases in sodium and increases in calcium, aluminum, and DOC concentrations were observed in all unit classes from baseflow to stormflow ($p<0.05$). The base cation sum was significantly higher in stormflow in all unit classes except for high elevation study sites (+4.79 μeq/L, +12.20 μeq/L for the five other classes). Magnesium significantly increased in low elevation waters (+2.44 μeq/L).

Strong linear relationships were exemplified between baseflow and stormflow pH and ANC concentrations in regression models. Modeling baseflow pH versus stormflow pH gave an r^2 value of 0.79 ($p<0.0001$): stormflow pH = -1.05 +1.12*(baseflow pH). The regression model for ANC had an r^2 value of 0.63 ($p<0.0001$): stormflow ANC = -8.86 + 1.20 * (baseflow ANC).

3.3 Relations between physical basin characteristics and water chemistry

The eight block-designed study sites exhibited unique chemistry signatures in baseflow and stormflow stream chemistry (Table 4). Eagle Rocks Prong, Rock Creek, and Road Prong had the lowest baseflow and stormflow pH and ANC concentrations, and the highest anion (SO_4, NO_3, and Cl) concentrations of the eight sites. In baseflow, pH was not significantly different between Newt, Lost Bottom, Jakes, Cosby, and Palmer (pH range: 6.50-6.67). These sites represent the low elevation sites (except Rock Creek) and the high elevation, no Anakeesta sites. The baseflow pH of Road Prong (6.30), Rock Creek (5.96), and Eagle Rocks Prong (5.39) were significantly different than the other five sites and each other. Similar differences among the study sites were observed with respect to pH in stormflow and to ANC in baseflow and stormflow (Table 4). Sulfate, nitrate, and cation concentrations were variable among the study sites, ranging from 18.82-50.90 μeq/L, 4.70-49.56 μeq/L, and 103.16-141.17 μeq/L in baseflow, and from 21.23-64.73 μeq/L, 4.41-52.15 μeq/L, and 110.13-150.14 μeq/L in stormflow.

ANOVA Tukey-Kramer HSD tests identified differences in baseflow and stormflow stream chemical constituents among block unit classes ($p<0.05$). High elevation sites had significantly lower pH, ANC, sodium, and silicon and higher nitrate concentrations than low elevation sites in baseflow and stormflow (Table 4). Additionally, high elevation sites had higher magnesium concentrations and anion sums (24.35 μeq/L, 77.07 μeq/L) than low elevation sites (20.98 μeq/L, 63.62 μeq/L) in baseflow; and higher aluminum and DOC concentrations and lower base cations (0.15 ppm, 3.47 mg/L, 120.79 μeq/L) than low elevation sites (0.08 ppm, 2.38 mg/L, 130.74 μeq/L) in stormflow. Small basin areas had significantly lower nitrate, sodium, magnesium, silicon, and base cation concentrations than large basin areas in baseflow and stormflow (Table 4). During stormflow, small basins also had significantly lower ANC and calcium concentrations (28.57 μeq/L, 55.22 μeq/L) than large basins (45.61 μeq/L, 65.00 μeq/L). Basins with greater than 10% Anakeesta area had significantly lower pH, sodium, and higher calcium concentrations than basins without Anakeesta geology in baseflow and stormflow (Table 4). During baseflow, basins with Anakeesta had lower ANC and silicon concentrations, and higher nitrate, sulfate, magnesium, and anion concentrations (28.29 μeq/L, 2.36 ppm, 25.20 μeq/L, 40.33 μeq/L, 23.68 μeq/L, 77.27 μeq/L) than basins without Anakeesta (41.45 μeq/L, 2.63 ppm, 16.97 μeq/L, 33.65 μeq/L, 21.55 μeq/L, 62.34 μeq/L). During stormflow, basins with Anakeesta had significantly higher concentrations of aluminum (0.15 ppm) than basins without Anakeesta (0.06 ppm).

The PCA analyses delineated groupings among study sites per chemical concentration loadings defining these groups (Figures 8-9). The first two principal components explained 85.1% (70.8%, 14.3%) of the variance in baseflow chemistries. The first three components explained 90.9% (58.8%, 19.4%, 12.6%) of the variance in stormflow chemistries. The baseflow PCA demonstrated four distinguishable groups: 1) low elevation, Anakeesta (Newt and Jakes) and high, elevation no Anakeesta (Palmer, Lost Bottom); 2) Cosby Creek; 3) Road Prong and Rock Creek; and 4) Eagle Rocks Prong. Groups 3 and 4 were distinguished from group 1 primarily due to lower pH and ANC, and higher sulfate and nitrate concentrations; and to a lesser extent because of higher base cations and DOC concentrations. Cosby Creek was distinguishable from group 1 primarily

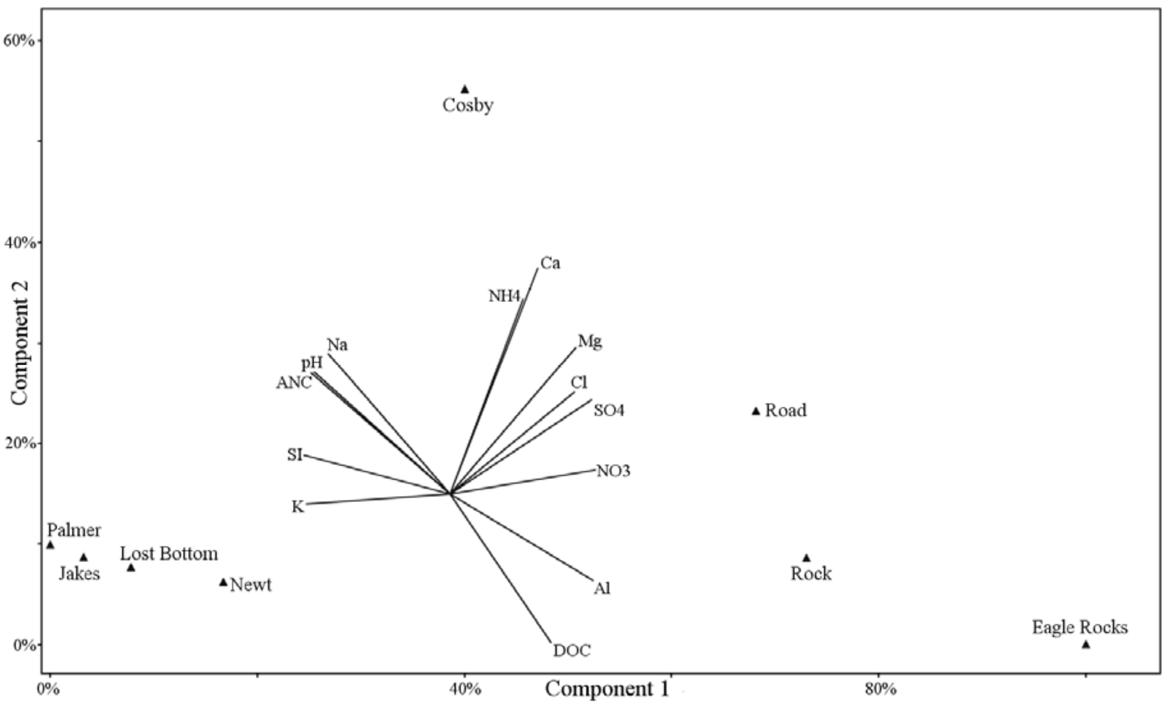

Figure 8: Loading plot and score plot of PCA of baseflow stream chemistry in block-designed study sites.

because of higher nitrate, sulfate, calcium, magnesium and sodium concentrations. During stormflow, Newt, Lost Bottom, Palmer, Jakes, and Cosby Creeks were grouped together; Road and Eagle Rocks Prong and Rock Creek differed from this group and each other. This group of low elevation sites (excluding Rock) and high elevation, no Anakeesta sites differed from the other three sites primarily because stormflow chemistries exhibited higher pH and ANC, sodium, and potassium and lower sulfate, nitrate, and aluminum concentrations.

Mean elevation, drainage area, percent area Anakeesta geology, mean basin slope, average soil saturated hydraulic conductivity and slope, and percent area with low elevation xeric forests and shrublands were correlated ($p<0.01$) with different baseflow and stormflow chemical constituents to different extents (Table 6). Site elevation was

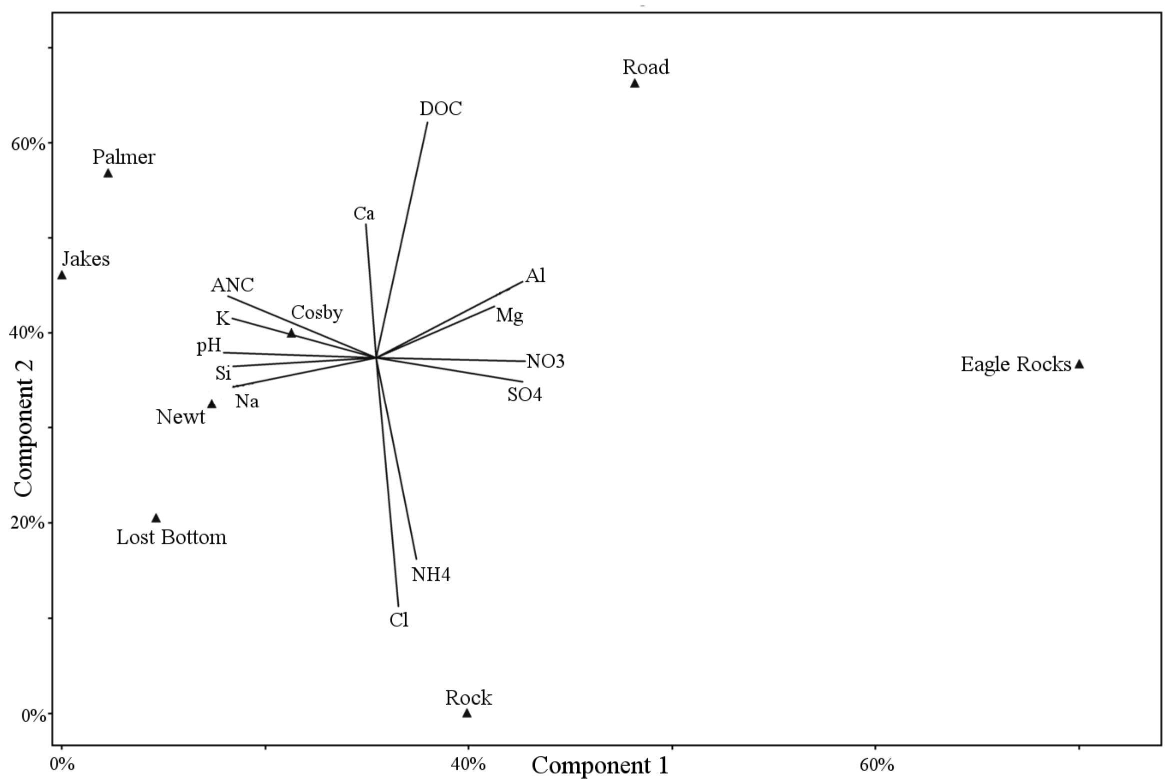

Figure 9: Loading plot and score plot of PCA of stormflow stream chemistry in block-designed study sites.

directionally correlated with the same chemical variables as mean basin slope, albeit not to as great as extent. During baseflow and stormflow, mean basin slope was positively correlated with sulfate (ρ=0.61, 0.57), nitrate (ρ=0.83, 0.65), magnesium (ρ=0.81, 0.79, 0.56), and aluminum (ρ=0.34, 0.42) concentrations, and negatively correlated with pH (ρ=-0.60, -0.50), ANC (ρ=-0.59,-0.55), sodium (ρ=-0.43, -0.47), and silicon (ρ=-0.58, -0.58) concentrations. Nitrate concentrations were negatively correlated with Anakeesta area in baseflow (ρ=-0.24) and stormflow (ρ=-0.25). In baseflow and stormflow, basin area was positively correlated with ANC (ρ=0.41, 0.32) and sodium (ρ=0.42, 0.24) concentrations, and negatively correlated with pH (ρ=0.40, 0.29). Calcium (ρ=0.38) and magnesium (ρ=0.39) concentrations were positively correlated with basin area in stormflow. Basin area also was positively correlated with silicon (ρ=0.35) and negatively correlated with sulfate (ρ=-0.27) in baseflow.

Chemical and physical soil characteristics including saturated hydraulic conductivity, soil pH, and soil slope, and percentage of forest types including low elevation xeric forests, subalpine mesic forests, and shrublands were more strongly correlated with baseflow and stormflow chemical constituents than the topographic (i.e. area, elevation, slope) and geologic (i.e. Anakeesta) basin factors (Table 6). The average soil organic percentage and soil depths, and high, low and mid elevation mesic forests had weaker correlations with stream chemistry variables. Area weighted average soil slope and saturated hydraulic conductivity were negatively correlated with pH, ANC, sodium, potassium, and silicon concentrations, and were positively correlated with nitrate, sulfate, aluminum, and magnesium concentrations; the percentage of low elevation xeric forests and shrublands had opposite correlations with these chemical constituents (Table 6). Soil pH was similarly correlated in significance (opposite direction) with chemical variables as saturated hydraulic conductivity. Likewise, the percentage of subalpine mesic forests was similarly correlated in significance (opposite direction) with chemical variables as low elevation xeric forests.

The CCA analyses demonstrated stream chemistry, in baseflow and stormflow, was strongly influenced by basin area-weighted soil chemical and physical properties (Figures 10-11). In baseflow, Axis 1 explained 79.9% of the variance in stream chemistry and Axis 2 explained 6.8% of the variance; in stormflow, Axis 1 explained 80.9% of the variance and Axis 2 explained 7.5% of the variance. Axis 1 was most influenced by soil pH and Ksat in both baseflow and stormflow whereas slope, organic percentage and soil depth

Table 6: Spearman correlation coefficients ($p<0.01$) for relationships between basin characteristics and baseflow (n=146) and stormflow (n=92) stream chemical constituents.

Chemical Constituent	Anakeesta (%)	Area (km^2)	Mean Elevation (m)	Mean Basin Slope (%)	Ksat (mg/L)	Soil Slope (%)	LEXF[A]	Shrub[B]
pH	– / –	0.40/0.29	-0.55/-0.53	-0.60/-0.50	-0.75/-0.62	-0.77/-0.72	0.78/0.67	0.41/0.38
ANC(μeq/L)	– / –	0.41/0.32	-0.53/-0.53	-0.59/-0.55	-0.74/-0.68	-0.75/-0.72	0.75/0.68	0.39/0.40
$N0_3$(μeq/L)	-0.24/-0.25	– / –	0.62/0.38	0.83/0.65	0.72/0.52	0.78/0.54	-0.77/-0.55	-0.75/-0.60
SO_4(μeq/L)	– / –	-0.27/ –	0.35/0.26	0.61/0.57	0.83/0.66	0.54	-0.80/-0.65	-0.49/-0.46
Cl(μeq/L)	– / –	– / –	– / –	0.19/ –	0.32/ –	– / –	-0.30/ –	-0.19/ –
Anions(μeq/L)	– / –	-0.24/ –	0.49/0.29	0.73/0.60	0.86/0.66	0.67	-0.86/-0.66	-0.58/-0.49
Na(μeq/L)	– / –	0.42/0.24	-0.54/-0.57	-0.43/-0.47	-0.73/-0.64	-0.66/-0.64	0.71/0.63	0.37/ –
Ca(μeq/L)	– /0.25	– /0.38	– / –	– / –	0.52/ –	0.20/ –	-0.48/ –	-0.57/-0.32
K(μeq/L)	– / –	– /0.22	– / –	-0.19/-0.23	-0.28/-0.42	-0.21/-0.35	0.28/0.43	– / –
Mg(μeq/L)	– / –	– /0.39	0.41/ –	0.79/0.56	0.62/0.34	0.56/0.26	-0.67/-0.39	-0.74/-0.57
Cations(μeq/L)	– / –	0.33/0.43	-0.26/ –	0.48/ –	– / –	– /-0.29	-0.32/ –	-0.55/ –
Al(ppm)	– / –	– / –	0.29/0.40	0.34/0.42	0.40/0.47	0.40/0.46	-0.43/-0.48	-0.28/-0.36
Si(ppm)	– / –	0.35/ –	-0.54/-0.60	-0.58/-0.58	-0.77/-0.59	-0.69/-0.64	0.76/0.58	0.47/0.51

[A] Low and mid elevation subxeric to xeric forests and woodlands.

[B] Shrublands or shrub understory.

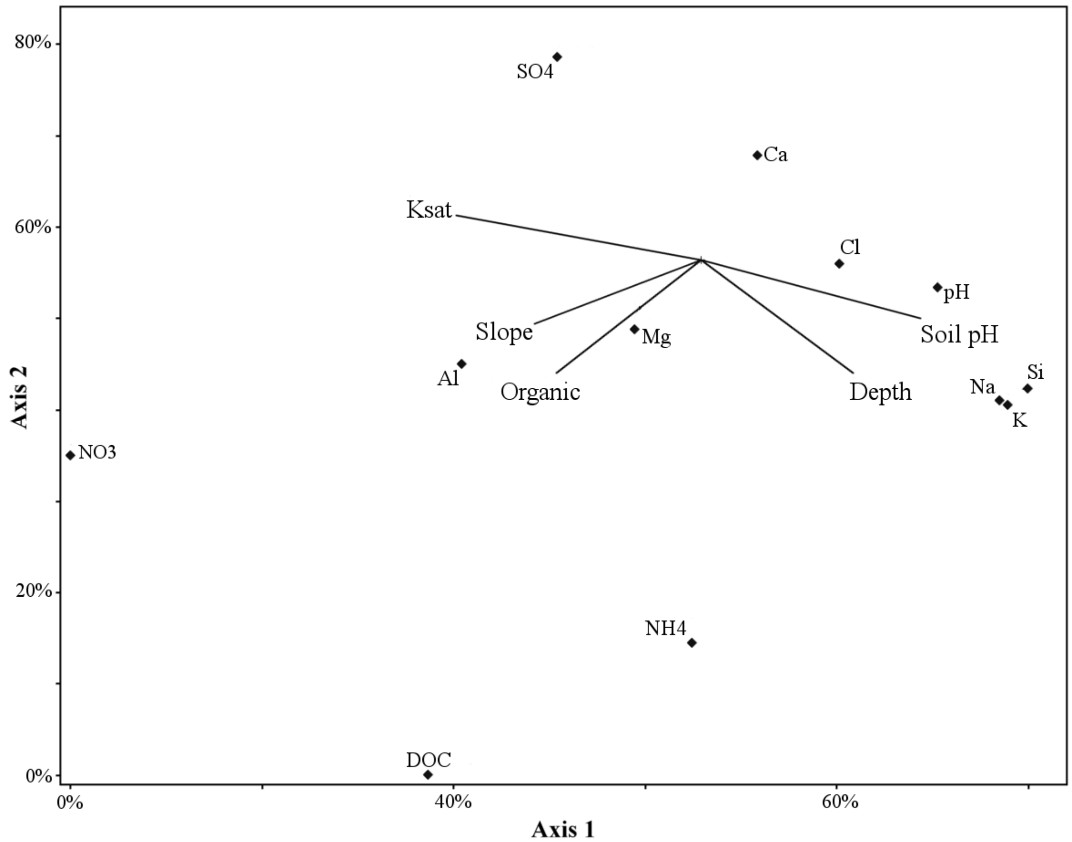

Figure 10: Biplot of the CCA model of baseflow water chemistry with environmental chemical and physical soil variables (weighted averages per basin area). Biplot cutoff R^2 values are 35% for soil variables displayed.

contributed to both axes. In baseflow stream chemistry, nitrate, aluminum, and DOC concentrations were positively influenced by soil slope and organic percentage; the concentration of calcium was negatively correlated to these soil properties. In basins with average shallower soils, sulfate concentrations were higher. Higher soil pH, deeper soil, and lower soil Ksat averages were associated with higher baseflow pH, sodium, potassium, and silicon concentrations. Stormflow chemistries were correlated similarly with soil variables with few exceptions. Stormflow DOC concentrations were not well correlated with soil variables. Nitrate concentrations in stormflow were higher in basins with higher organic percentages and hydraulic conductivities. Higher aluminum and sulfate

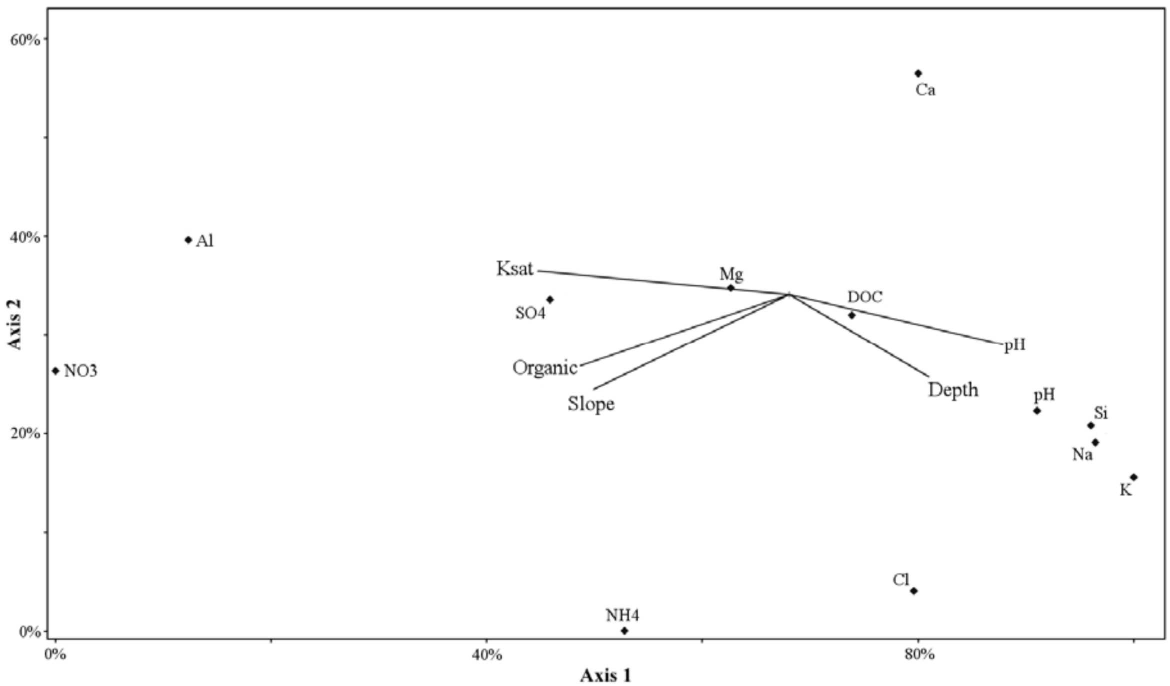

Figure 11: Biplot of the CCA model of stormflow water chemistry with environmental chemical and physical soil variables (weighted averages per basin area). Biplot cutoff R^2 values are 35% for soil variables displayed.

concentrations were associated with lower soil pH and higher Ksat concentrations.

Stream chemistry in baseflow and stormflow was also influence by the vegetation composition in basins (Figures 12-13). In baseflow, Axis 1 explained 81.4% of the variance and Axis 2 explained 7.5% of the variance in stream chemistry; in stormflow, Axis 1 explained 84.9% of the variance and Axis 2 explained 6.7% of the variance. Axis 1 was primarily influenced by SAMF, LEXF, LEMF, and shrub; Axis 2 was most influenced by HEMF. Nitrate concentrations were higher in basins dominated by sub-alpine and high elevation forests and lower in basins with higher percentages of shrub and LEMF vegetation types. Greater HEMF in basins was also associated with higher aluminum, ammonium and DOC concentrations, and lower calcium concentrations. In basins with higher percentages of LEXF forests, baseflow pH, sodium, silicon and potassium

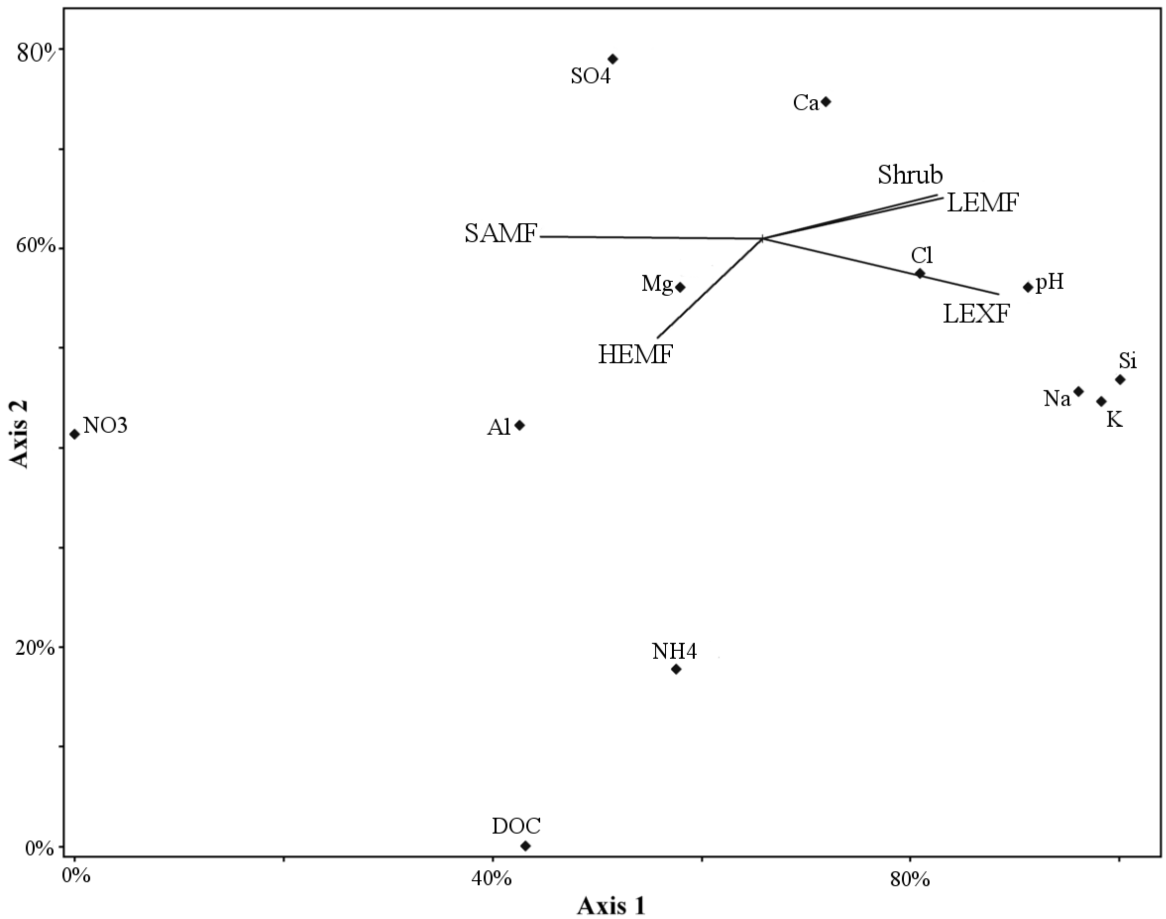

Figure 12: Biplot of the CCA model of baseflow water chemistry with environmental vegetation variables (area percentage in basin: SAMF – sub-alpine mesic forests and woodlands, HEMF – high elevation mesic to submesic forests, LEMF – low and mid elevation mesic to submesic forests, LEXF – low and mid elevation subxeric to xeric forests and woodlands, and Shrub – shrublands or shrub understory). Biplot cutoff R^2 values are 35% for soil variables displayed.

concentrations were higher, and sulfate concentrations were lower. In stormflow, sub-alpine forests related to higher sulfate, nitrate, and aluminum concentrations and lower pH, silicon, sodium, and potassium concentrations. DOC concentrations were not strongly associated with vegetation types in stormflow.

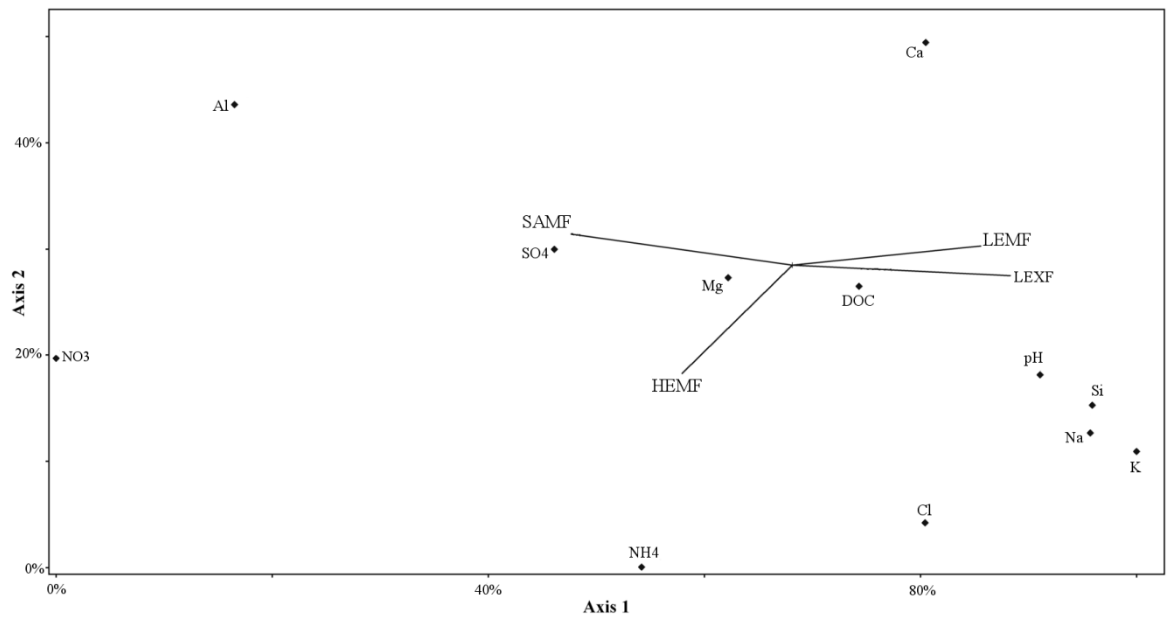

Figure 13: Biplot of the CCA model of stormflow water chemistry with environmental vegetation variables (area percentage in basin: SAMF – sub-alpine mesic forests and woodlands, HEMF – high elevation mesic to submesic forests, LEMF – low and mid elevation mesic to submesic forests, LEXF – low and mid elevation subxeric to xeric forests and woodlands, and Shrub – shrublands or shrub understory). Biplot cutoff R^2 values are 35% for soil variables displayed.

3.4 Stepwise multiple regressions

Predictive stormflow pH and ANC models from baseflow chemistry regressors were produced (Table 7). The best model to predict stormflow pH was simply the linear regression of baseflow pH with stormflow pH reported previously. When other variables were added to this model, the intercept became insignificant or there were issues with multicollinearity. The predictor variables for stormflow ANC, including baseflow pH, chloride and sodium concentrations, produced a model with an adjusted r^2 of 0.64. Higher stormflow ANC concentrations are predicted in waters with higher baseflow pH and sodium concentrations and lower chloride concentrations.

Table 7: Stepwise linear regression results.

Stormflow Constituent		Basin Regressors		Baseflow Chemical Constituent Regressors							
	Intercept	Site Elevation (km)	Mean Slope (%)	pH	ANC(μeq/L)	Cl(μeq/L)	Cations(μeq/L)	Na(ppm)	n	Adjusted r^2	RMSE
pH	-1.05			1.12					90	0.79	0.27
ANC(μeq/L)	-163.8			30.02		-5.15		2.16	89	0.69	17.2
pH	1.09	-0.69		1.09					90	0.84	0.24
ANC(μeq/L)	133.5		-4.73		0.77	-5.28	0.53		89	0.75	16.6

Combined models improved the predictability of the stepwise regression models (Table 7). The stormflow pH model was improved by adding site elevation as a basin regressor to complement the baseflow pH regressor in the chemistry regression model. The model improved the adjusted r^2 to 0.84 and reduced the RMSE from 0.27 to 0.24. Lower stormflow pH is predicted with higher site elevations and lower baseflow pH values. The model to predict stormflow ANC improved in the combined approach with basin regressor mean slope and baseflow chemistry regressors ANC, chloride, and cation concentrations (r^2 = 0.74, RMSE = 16.8). Lower ANC concentrations are predicted in basins with greater slopes that have lower baseflow ANC and cation concentrations and higher chloride concentrations.

4. Discussion

Interrelated basin characteristics including elevation, basin area, Anakeesta geology and soil and vegetation influenced baseflow and stormflow chemistry in GRSM basins. As in other studies, this research demonstrated relationships between baseflow and stormflow chemistry (Deviney et al., 2006; Rice et al., 2004) and basin-scale factors governing local chemical processes that impact stream chemistry and resultant acidification response (Clow and Sueker, 2000; Likens and Buso, 2006; Sullivan et al., 2007). Increases in aluminum concentrations were associated with pH depressions in stormflow most likely because of increased solubility of aluminum in decreasing pH waters (Driscoll and Postek, 1995). The concentrations of DOC increased during stormflow at all of the study sites, suggesting organic acids also contributed to the acidification response (Deyton et al., 2009; Driscoll et al., 1989).

This research provides further evidence that elevation is a controlling factor in acidification response in GRSM streams because at higher elevations there are higher rates of acid deposition, precipitation and base cation leaching; and steeper slopes in less well-developed soils (Deviney et al., 2006; Weathers et al., 2006). Interestingly, mean basin slope, highly correlated with site elevation, had stronger associations with baseflow and stormflow chemistry than elevation. This is likely because slope also relates the processes of flowpaths and residence times in shallow soils influencing stream chemistry (McGuire et al., 2005).

At the spatial scale defined in this study (basin areas less than 20 km^2), basin area had less influence in predicting stormflow chemistry than elevation, although streams with larger basin areas had higher nitrate, sodium, and base cation concentrations in baseflow and stormflow, and higher ANC and calcium concentrations in stormflow. This suggests that streams with smaller basin areas have reduced subsurface contact time and subsequently are more acidic and have lower ANC concentrations (Deyton et al., 2009; Wolock et al., 1997).

Weight-averaged soil parameters among soil types and horizons within basins enable clear relationships between soil characteristics and stream chemistry. Important soil parameters related to stream chemistry in this study control water pathways through basins and the length of time water resides in the soil (Wolock et al., 1989). Similar to what was reported by Wolock et al. (1989), Ksat, of all the soil parameters, was the soil characteristic most related to concentrations of stormflow chemical constituents. Higher hydraulic conductivities were associated with lower pH, ANC, and base cation concentrations, and consequent higher nitrate and sulfate concentrations from flushed acid deposition constituents and higher aluminum concentrations from increased solubility (Driscoll and Postek, 1995). Although predicting concentrations of ionic species in stormflow is difficult because hydrologic pathways change and water flows through multiple soil horizons (Billett and Cresser, 1992), deeper soils were associated with higher sulfate concentrations (Shanley, 1992), and higher organic percentages were related to higher nitrate concentrations in stormflow (Mulholland, 1993).

Although the percentage of forest types in basins contributed to the chemical signatures of the study streams, forest types are dependent on elevation, climate and soil characteristics (Day et al., 1988). In this study, nitrate was higher in basins dominated by sub-alpine and high elevation forests and was lower in basins with high percentages of shrub and low elevation mesic forests. These results suggest biogeochemical processes including nitrification and mineralization may impact stream chemistry from increased organic acid and nitrate concentrations (Andersson and Nyberg, 2009; Mulholland, 2004). Coupled with higher rates of acid deposition (Weathers, et al., 2006), steeper slopes, and thinner more acidic soils (USDA-NRCS, 2009), streams dominated by high elevation forests were also associated with higher sulfate, DOC and aluminum concentrations and lower pH, and base cation concentrations in stormflow.

In this research, we wanted to test if undisturbed Anakeesta geology impacted stream acidification. We limited the scope of the effects of geology on water chemistry to presence of Anakeesta, and excluded basins with limestone geology which buffer streams from acid inputs (Sullivan et al., 2007). Although Anakeesta geology was associated with stream chemistry, this may be a circumstantial result because the two high elevation Anakeesta sites also had the steepest slopes, highest mean basin elevations, lowest soil pH, and the greatest percentage of sub-alpine and high elevation mesic forests, which contributed to greater acidification in these streams.

Although depressed pH in stormflow was demonstrated in this study, significant reductions in ANC concentrations only occurred in Eagle Rocks Prong. In Rock Creek, whose baseflow pH was significantly lower than the other sites except Eagle Rocks Prong, the pH did not decrease in stormflow. Rock Creek differed in its response to precipitation events in having significantly higher base cations in conjunction with anion dilution in stormflow. In the other basins that experienced little or no difference in ANC concentrations yet had proton concentration increases, the average baseflow pH (6.60) was greater than that of precipitation pH (4.60; NADP, 2009), and base cations and anions increased from baseflow to stormflow. Base cations were lower in baseflow than stormflow at all sites except at Eagle Rocks and Road Prongs, demonstrating the acidification response was primarily related to elevation, slope, soil, and vegetative characteristics at these sites.

Examining the effects of elevation, area, geology, soil, and vegetation on stream chemistry supports the management of aquatic resources from potential acid deposition impairment in the GRSM. With the increased availability of digital topographical, geological, pedological, and vegetative spatial databases, it is now practical to systematically analyze relationships between stream chemistry and a broad range of basin characteristics (Allan, 2004). A simple approach directly relating measurable variables and basin characteristics enables prediction of concentrations of ionic species in streams, critical to the health and survival of aquatic organisms (Billett and Cresser, 1992). This research demonstrates that models involving linear regressions of basin characteristics on water chemistry as predictive tools can be useful despite not simulating hydrochemical processes (Clow and Sueker, 2000). Using GIS and digital databases in conjunction with water quality data can provide a less time consuming and less expensive way to predict water chemistry and evaluate water chemistry to facilitate management of water resources (Sullivan et al., 2007).

Acknowledgements

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Chapter IV: Physiological Stress in Native Southern Brook Trout during Episodic Stream Acidification in the Great Smoky Mountains National Park

This chapter is revised based on a paper published by Keil J. Neff, John S. Schwartz, Theodore B. Henry, R. Bruce Robinson, Stephen E. Moore, and Matt A. Kulp.

My primary contributions to this paper included (i) developing methods to accomplish study objectives, (ii) selecting sites and installing monitoring equipment, (iii) conducting field experiments and laboratory analyses, (iv) analyzing data, (v) pulling contributions into a single paper, and (vi) primarily authoring the paper.

Neff, K. J., Schwartz, J. S., Henry, T. B., Robinson, R. B., Moore, S. E., and Kulp, M. A. (2009). "Physiological stress in native southern brook trout during episodic stream acidification in the Great Smoky Mountains National Park." Archives of Environmental Contamination and Toxicology, 57(2), 366-376.

Abstract

Episodic stream acidification from atmospheric deposition is suspected to detrimentally impact native southern brook trout (*Salvelinus fontinalis*) in Great Smoky Mountains National Park (GRSM) headwater streams. To test the hypothesis that

episodes of stream acidification cause physiological distress to native trout, caged fish at three sites were exposed to acid episodes during in situ bioassays conducted in June 2006 and March 2007. Stream pH decreased (> 0.7 pH units) and total dissolved aluminum (Al_{TD}) increased (> 175 μg/L) at all three sites during acid episodes in both bioassays. Whole-body sodium concentrations were significantly reduced (10-20%) following the acid episodes when preceding 24-h mean pH values (4.88, 5.09, 4.87) and corresponding 24-h time weighted average Al_{TD} concentrations (210, 202, 202 μg/L) were observed. Lower whole-body sodium concentrations were correlated with elevated H^+ and Al_{TD} concentrations. Loss of sodium ions in native southern brook trout were consistent with physiological distress resulting from acid exposure reported in salmonids in other investigations. Further research is necessary to conclude whether acid episodes are responsible for extirpation of brook trout from headwater streams in the GRSM.

Acknowledgements

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Chapter V: The Influence of Chemical, Hydrological, and Basin Factors on Brook Trout (*Salvelinus fontinalis*) in Streams of the Great Smoky Mountain National Park, USA

This chapter is revised based on a paper to be published by Keil J. Neff, John S. Schwartz, Stephen E. Moore, Matt A. Kulp, and Theodore B. Henry.

My primary contributions to this paper included (i) developing problem into a work, (ii) identifying study objectives, (iii) acquiring and assembling data sets, (iv) analyzing data, (v) pulling contributions into a single paper, and (vi) primarily authoring the paper.

Neff, K. J., Schwartz, J. S., B., Moore, S. E., Kulp, M. A., and Henry, T. B., (2010). "The Influence of Chemical, Hydrological, and Basin Factors on Brook Trout (*Salvelinus fontinalis*) in Streams of the Great Smoky Mountain National Park, USA." Environmental Pollution, *in preparation for submittal*.

Abstract

In streams of the Great Smoky Mountains National Park, brook trout densities and condition factor, K, values were evaluated with respect to baseflow water chemistry, basin characteristics, and hydrology. Sixteen collocated sites (allopatric brook and fishless), with fish field survey data (1990-2009) and water quality monitoring data (1994-2009) were considered for study. Flows were modeled from 1990-2007 at these sites using WinHSPF; Indicators of Hydrologic Alteration were computed to characterize daily flows into ecologically relevant parameters. Several statistical methods were employed to investigate relationships between brook trout population metrics, and chemical, hydrological and basin variables; compare spatial and temporal variation in and among sites; and explain which factors explained the greatest proportion of variability in trout densities. Basin factors accounted for the greatest proportion of variability in young-of-year (YOY) and adult brook trout densities. Adult brook trout densities were positively correlated ($p<0.05$) with elevation and average soil cation exchange concentration. Spatial variability was greater than temporal variability in trout populations, and temporal variability in YOY populations was more than double the variability in adult populations. This suggests that YOY trout may be more susceptible to hydrologic disturbances or episodes of stream acidification. Higher concentrations of ANC, sodium and pH were associated with the presence of brook trout. Trout densities were higher in streams with higher concentrations of sodium, suggesting that, at the population level, sodium may ameliorate the effects of acid toxicity. Fall flows were positively correlated ($p<0.05$) with total brook trout densities and YOY trout densities were significantly lower when there was a flood within one year of the sampling date. This study demonstrates the interaction of chemical, hydrological, and basin factors influencing brook trout distributions and densities.

1. Introduction

Abiotic and biotic factors influence the abundance and distribution of brook trout (*Salvelinus fontinalis*) in stream networks throughout the eastern United States (Bulger et al., 2000; Kocovsky and Carline, 2005; Strange and Habera, 1998). The coupled interaction of chemical, hydrological, and basin factors may influence brook trout populations in Great Smoky Mountains National Park (GRSM) streams (Franco and Budy, 2005; Hudy et al., 2008).

Atmospheric acid deposition contributes to chronic and episodic acidification in poorly buffered GRSM streams potentially threatening native brook trout (Deyton et al., 2009; Neff et al., 2009; Robinson et al., 2008). Brook trout can die from exposure to elevated proton and monomeric inorganic aluminum concentrations resulting from stream acidification (Baldigo et al., 2007; MacAvoy and Bulger, 2004) by disturbing gill ion transport (Booth et al., 1988) or causing asphyxia (Neville and Campbell, 1988). Additionally, episodic stream acidification can cause sublethal distress in fish (Neff et al., 2009) resulting in downstream immigration (Gagen et al., 1993), less successful reproduction (Kaeser and Sharpe, 2001), impaired swimming (Wilson and Wood, 1992), and decreased growth (Cleveland et al., 1991; Mount et al., 1988). As with most fish, early life stages of brook trout are more acid sensitive than older ones (Baldigo and Lawrence, 2001).

Basin factors influence the distribution of trout by affecting water chemistry and habitat factors at the reach scale (Kocovsky and Carline, 2005). Research has demonstrated that trout populations are influenced by basin and channel factors including habitat patch area (Rieman and McIntyre, 1995), geology (Bulger et al., 2000; Kirby et al., 2008), soil-water interaction (Swistock et al., 1997), stream gradient (Budy et al., 2008;

Lanka et al., 1987), and in-stream habitat components and stream morphology (Jackson et al., 2001; Kozel and Hubert, 1989). Trout distribution is also regulated by stream temperature (Jackson et al., 2001; Meisner, 1990) and discharge (Lobon-Cervia, 2004; Shuter et al., 1980), which are governed by hydro-climate, elevation, canopy cover, and other basin characteristics. In the GRSM, 82% of native brook trout inhabit stream above 914 m (Moore and Kulp, 2010). A gradient exists in the GRSM such that with increasing elevation, streams have less buffering capacity and resultant lower baseflow and stormflow pH (Robinson et al., 2008).

Hydrological processes impact the distribution, diversity, and abundance of riverine species (Lake, 2000; Poff and Allan, 1995). Stream ecological integrity is influenced directly and indirectly by natural flow regime components of magnitude, frequency, duration, timing, and rate of change (Poff et al., 1997). The natural flow regime can affect abiotic characteristics such as flow depth and velocity, temperature, oxygen content, turbidity, streambed substrate, and morphology (Richter et al., 1997). In relation to flow disturbances (floods or droughts), flow regime components may dictate the success or failure of populations in stream ecosystems (Lake, 2000). Floods may cause rapid effects on fish populations primarily from high in-stream velocities and debris flow, which may cause death, displacement, reduce effective habitat, or damage fish eggs and/or larvae in redds (Carline and McCullough, 2003; Elwood and Waters, 1969). Severe flooding in salmonid-dominated streams commonly destroys the year class of fish that are still incubating or have recently emerged and can take two to three years to recolonize (Carline and McCullough, 2003). Drought reduces habitat leading to increased fish density, and thereby increasing biotic interactions of predation and competition for diminishing food

(Closs and Lake, 1996; Cowx et al., 1984; Matthews and Marsh-Matthews, 2003). Low water levels may impact fish health due to altered water quality (particularly temperature and oxygen); or reduce suitable spawning habitat, thereby leading to possible loss of a year class (Lake, 2003; Richter et al., 1996).

With a majority of brook trout populating higher elevation streams that are more susceptible to acidification, it is important to understand the relative sensitivity of native brook trout to stream chemistry, basin and channel characteristics, and hydrologic flow regimes in GRSM streams. The objectives of the study were to: 1) evaluate brook trout densities and condition factor, K, values with respect to baseflow water chemistry, physical basin characteristics, and hydrology, and 2) assess the relative importance of the chemical, basin, and hydrology variables to annual variation in brook trout densities.

2. Methods

2.1 Study area

There are 3,379 km of streams in the 2,108 km^2 GRSM, located in the Blue Ridge physiographic region of eastern Tennessee and western North Carolina (Figure 14). Altitudes in the GRSM range from 300 m to 2,025 m. GRSM basins are heavily forested with steep gradients and thin sandy loams that provide poor buffering capacities. Streambeds are dominated by boulder and cobble, and channel slopes increase with elevation (Larson and Moore, 1985). The climate of GRSM is perhumid mesothermal with

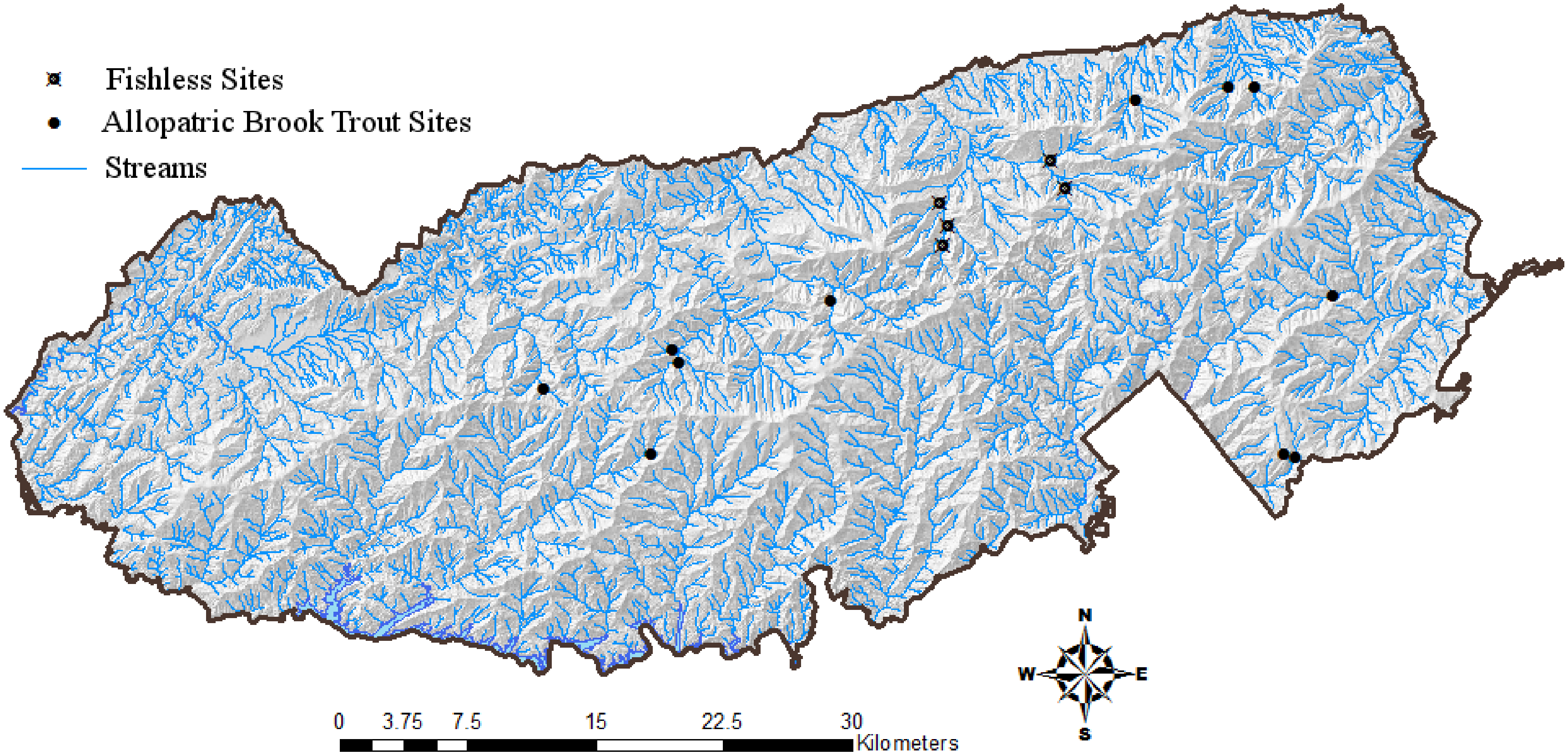

Figure 14: Location of collocated water chemistry and trout sample sites in the Great Smoky Mountains National Park. Populations were considered marginal when densities were less than 0.5 pop/dm^2 and less than 50% of field surveys yielded YOY or adult trout. Chemistry sites with no brook trout were located upstream of fish surveys without fish and without downstream fish barriers preventing colonization.

seasonal temperature variation and precipitation distributed throughout the year (Busing, 2005). The average annual rainfall varies significantly throughout the park with lower elevations generally receiving near 127 cm and some higher elevation sites near 216 cm (Busing, 2005).

Trout species found in the GRSM include brook trout, and introduced rainbow (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) species. Brook trout are the only salmonid species indigenous to the southern Appalachian Mountains (King, 1939); rainbow trout were introduced into GRSM streams in the early 1900's (Larson et al., 1995). Brook trout are regulated to headwater streams because of historical logging and competition from rainbow trout in larger streams (Larson and Moore, 1985).

2.2 Data

Sixteen collocated allopatric brook trout (n=11) trout and fishless (n=5) stream sites, with fish field survey data and water quality monitoring data were considered for study (Figure 14). Fish data used in this study was collected by GRSM fishery biologists from 1990-2009. Trout were collected from 100 m representative stream reaches by standard three-pass depletion electroshocking, measured for total length (mm) and weight (g), and separated into two size groups, young-of-year (YOY) and adults, using length frequency delineation (Reynolds, 1996). Population estimates were generated with Microfish 3.0 (Van Deventer and Platts, 1989) using the maximum-likelihood model (Platts, 1983). Trout densities were calculated as population per unit area of stream (number of fish/dm^2) using stream measurements of length and average width (measured in 10 m intervals).

Condition factor, K ($K=W/L^3$), was calculated for each specimen (Reynolds, 1996); an average K was applied to each sample site for each sampling date as a qualitative assessment of health (Fulton, 1911).

Long-term synoptic baseflow stream water quality monitoring began in 1993 to characterize stream chemistry in GRSM streams and monitor long-term trends (Robinson et al., 2008). Sites were selected to assess the spatial variability of water quality within the GRSM across a range of elevations, geology types, and disturbance histories. From 1993 to the present, the number of sites has been reduced from 367 to 43; sampling frequencies have varied throughout the period. Water chemistry parameters measured from 1993-2002 included pH, ANC, conductivity, sulfate, nitrate, chloride, ammonium, sodium, potassium, calcium, magnesium. Measurements of aluminum, zinc, copper, iron, manganese, and silica were added to laboratory analyses in the fall of 2002. Detailed laboratory methods can be found in Deyton et al. (2009) and Robinson et al. (2008). Baseflow chemistry data from 1994-2009 was included in this study.

The Hydrological Simulation Program - FORTRAN (WinHSPF) was used to model flows of collocated sites for the period 1990-2007 (Parker, 2008). Seven different climate layers were simulated in the GRSM using time series data from 16 weather stations (managed by the Tennessee Valley Authority, the National Oceanic and Atmospheric Administration, or the GRSM). Models were calibrated by adjusting storage, infiltration, runoff, and groundwater parmaters for three elevation classes (low < 800 m $\leq$ medium < 1200 m $\leq$ high) to fit flow output from model with two USGS gaging stations and one NPS gaging station. Following US EPA recommendations, acceptable performance of calibrated models were verified and met stipulated criteria (Donigian et al., 1983; Parker, 2008; USEPA, 2000).

Indicators of hydrologic alteration (IHA) were computed using IHA software developed by the Nature Conservancy for modeled flows of collocated sites to characterize daily flows into ecologically relevant parameters (Richter et al., 1996). Hydrologic variation in the flow regime of each site was characterized into 32 parameters of five hydrological groups (flow parameters of magnitude, timing, frequency, duration, and rate of change). IHA variables chosen to represent hydrological extremes included the 10-year flood, the two largest 2-year floods, and the two most extreme 90-day minimum flows.

ArcGIS® 9.3 (Environmental Systems Research Institute) was utilized to determine basin characteristics using a 10-meter Digital Elevation Model (USGS), and digital geological (Southworth et al., 2004), soil (USDA-NRCS, 2009), and vegetation (Madden et al., 2004) maps. ArcHydro tools (Center for Research in Water Resources) and Spatial Analyst tools were used to delineate basins and compute basin areas, elevations, and slopes, and channel slopes and lengths; the Spatial Analyst Zonal Statistics Tool enabled detailed physiographic, geological, soil and vegetation information to be computed for each basin.

General soil characteristics, including soil reaction (soil pH), effective cation exchange capacity (CEC), organic percentage, saturated hydraulic conductivity (Ksat), average soil depths, and average soil slopes, were applied to each basin. This was accomplished by calculating weighted averages for each soil parameter and computing an area weighted average of these parameters for each basin. Vegetative characteristics were summarized in study basins by calculating the percent area of the dominant over-story vegetation forest types in each basin. Jackson et al. (2004) provides a complete description of these forest types.

2.3 Statistical analyses

Pearson pairwise correlation analyses were performed to investigate the relationships between YOY, adult and total trout densities in sites with brook trout populations. Relative standard errors (RSE) of YOY and adult brook trout densities were calculated for each of the collocated sites to compare the variability between the two age classes and to assess temporal variation within these age classes. Similarly, the RSE of average trout densities among collocated sites were calculated as a measure of spatial variation. Following the approach of Kocovsky and Carline (2005), the relative importance of spatial versus temporal variation was assessed by comparing the temporal and spatial relative standard errors.

Analysis of variance (ANOVA) Tukey honestly significant difference (HSD) tests were used to test differences in hydrology, basin characteristics, and baseflow chemistry between allopatric brook trout (n=11) and fishless sites (n=5). Chemical, hydrological, and basin variables are listed in Table 8. Spearman bivariate correlation analyses were performed in order to investigate the relationships between average brook trout densities, condition factor, K, values, and temporal variability (RSE), with chemical, hydrological and basin variables in brook trout streams (n=11). Tukey HSD tests were also used to test differences in trout densities between range classes of significant independent. Considering the trout densities sampled from each of the brook trout sites during the period

Table 8: List of variables for analyses. Italicized variables indicate categorical variables.

Basin Variables (N=22)	**Description/Units**
Site Elevation	Meters
Basin Area	Square kilometers
Max Basin Elevation	Meters
Mean Basin Elevation	Meters
Stream Order	Stream order
Channel Slope	%, USGS method
Longest Flow Path	Kilometers, USGS method
10%-85% Elevation Dif.	Meters, USGS method (reference)
Anakeesta Area	Square kilometers
Anakeesta %	Percent of basin with Anakeesta geology
Mean Slope	Average basin slope (%)
Soil pH	Area-weighted soil reaction rate (pH units)
Soil CEC	Area-weighted soil cation exchange capacity (meq/100g)
Soil Organic	Area-weighted percent organic in soil
Soil Ksat	Area-weighted soil hydraulic conductivity (cm/hr)
Soil Depth	Area-weighted subsoil depth (cm)
Soil Slope	Area-weighted soil slope (%)
SAMF	Percent area sub-alpine mesic forests and woodlands
HEMF	Percent area high elevation mesic to submesic forests
LEMF	Percent area low and mid elevation mesic to submesic forests
LEXF	Percent area low and mid elevation subxeric to xeric forests and woodlands
Shrub	Percent area shrublands or shrub understory
Hydrology Variables (N=24)	
Median Q (1990-2007)	Median flow (cms) from 1990-2007
"Month" Q	Median value for each calendar month (expressed as fraction of annual Q)
Reversals	Number of hydrologic reversals
Median Q (prior WY)	Median flow (cms) prior water year to fish sample date
Max Q (S,O,N; prior Y)	Maximum flow (cms) prior year to fish sample date (September-November)
Median Q (S,O,N; prior Y)	Median flow (cms) prior year to fish sample date (September-November)
Min Q (S,O,N; prior Y)	Minimum flow (cms) prior year to fish sample date (September-November)
Max Q (M,A,M; prior Y)	Maximum flow (cms) prior year to fish sample date (March-May)
Drought	Trout sampled during period of drought
Drought (prior Y)	Trout sampled within one year of drought
Drought (Prior 3Y)	Trout sampled within three years of drought
Flood (prior Y)	Trout sampled within one year of flood
Flood (Prior 3Y)	Trout sampled within three years of flood

Table 8 (continued)

Chemistry Variables (N=24)	
AVG pH	Average pH
AVG ANC	Average ANC (μeq/L)
AVG Cl	Average chloride (μeq/L)
AVG NO3	Average nitrate (μeq/L)
AVG SO4	Average sulfate (μeq/L)
AVG Anions	Average anion sum (μeq/L)
AVG Na	Average sodium (μeq/L)
AVG NH4	Average ammonium (μeq/L)
AVG K	Average potassium (μeq/L)
AVG Mg	Average magnesium (μeq/L)
AVG Ca	Average calcium (μeq/L)
AVG Cations	Average cation sum (μeq/L)
1YR pH	Average pH, year prior to fish sample date
1YR ANC	Average ANC (μeq/L), year prior to fish sample date
1YR Cl	Average chloride (μeq/L), year prior to fish sample date
1YR NO3	Average nitrate (μeq/L), year prior to fish sample date
1YR SO4	Average sulfate (μeq/L), year prior to fish sample date
1YR Anions	Average anion sum (μeq/L), year prior to fish sample date
1YR Na	Average sodium (μeq/L), year prior to fish sample date
1YR NH4	Average ammonium (μeq/L), year prior to fish sample date
1YR K	Average potassium (μeq/L), year prior to fish sample date
1YR Mg	Average magnesium (μeq/L), year prior to fish sample date
1YR Ca	Average calcium (μeq/L), year prior to fish sample date
1YR Cations	Average cation sum (μeq/L), year prior to fish sample date
Biotic Variables (N=9)	
BKT YOY DENS	Brook trout young-of-year density (population/dm2)
YOY BKT (prior Y)	Brook trout young-of-year density (population/dm2); prior year
BKT ADT DENS	Brook trout adult density (population/dm2)
ADT BKT (prior Y)	Brook trout adult density (population/dm2); prior year
BKT TOT DENS	Brook trout total density (population/dm2)
YOY BKT K	Young-of-year brook trout k-factor
ADT BKT K	Adult brook trout k-factor
Total BKT K	Total brook trout k-factor
BKT K (prior Y)	Total brook trout k-factor; prior year

of record (n=162), Tukey-Kramer HSD tests were performed to test differences in YOY and adult trout densities, and condition factors between categorical classifications of hydrological disturbance variables listed in Table 8.

Simple linear regression was utilized to explain which chemical, hydrological, biotic, and basin factors explain the greatest proportion of variability in brook trout densities and condition factor. Dependent variables consisted of adult and YOY trout densities, and K (condition factor) values; independent variables are listed in Table 8. The r^2 and directional relation between regressor and independent variable were reported for the two models in each factor class (hydrology, basin, chemistry, biotic) with the highest r^2 values.

Using stepwise multiple regression, adult and YOY brook trout densities were modeled utilizing hydrological, basin and chemical predictive variables. Only significant models ($p<0.05$) with associated significant independent variables and intercepts ($p<0.05$) were considered. Akaike's Information Criterion (AIC) and Mallows C_p were minimized (Mallows, 1973). Multicollinearity was addressed using the variance of inflation factor (VIF) and informal multicollinearity diagnostics including Spearman bivariate correlations. Independent variables with VIF > 10 were removed in reverse order of the explanatory ability to produce the best models while minimizing multicollinearity. The simplest models explaining YOY and adult brook trout densities explaining the most variability were selected as the final models. The directional relation between regressor and independent variables, and r^2 were reported. The JMP platform (SAS Institute Inc.) was used for statistical analyses.

Principal components analysis (PCA) was used to examine the relations among collocated sites and explain the chemical, hydrological and basin variable loadings defining the relationship between these sites. Matrices for the PCA analyses comprised of four variables in each factor class: hydrology (median Q, reversals, October Q, February Q); chemistry (pH, ANC, SO_4, Cl); and basin (soil slope, Anakeesta area, site elevation, and soil CEC) of the collocated sites. Principal components were included for evaluation when more than 80% of the variance was explained by the model, and individual component eigenvalues were greater than one.

3. Results

During the 20-year period (1990-2009), brook trout populations at the study sites were sampled an average of 15 times (Table 9). The mean adult trout density (11.22 +/- 3.84 fish/dm^2) was greater than the mean YOY trout density (7.75 +/- 5.09 fish/dm^2) for all of the sites and within each site (Table 9). Pearson bivariate correlations between average YOY, adult and total trout densities (n=11) demonstrated strong correlation ($p<0.001$, YOY-adult $\rho=0.865$, YOY-total $\rho=0.951$, adult-total $\rho=0.978$). Taking into account the trout densities for all sampled years (n=162), correlations were also significant ($p<0.0001$, YOY-adult $\rho=0.582$, YOY-total $\rho=0.868$, adult-total $\rho=0.908$). Considering variation within each site, RSE were more than twice as high in YOY trout densities than in adult trout densities (Table 9). The temporal variation of total brook trout density (RSE) in the 12 sites ranged from 5.6% to 18.0% (mean = 9.58%), whereas the average spatial RSE was

Table 9: Simple statistics and relative standard errors (RSE) of YOY and adult brook trout densities for the 11 allopatric brook trout GRSM streams in this study (1990-2009). N is the number of sampling dates. K is the condition factor (10^5*W(g)/L(mm)^3). Trout density expressed as fish per dm^2; RSE expressed as %.

Site	ID	N	K	YOY Density					Adult Density				
				Min	Mean	Max	SD	RSE	Min	Mean	Max	SD	RSE[3]
Ashe Camp	ACB	3	0.86	5.63	9.24	14.53	4.68	29.28	5.45	7.50	9.50	2.03	7.12
Bunches Creek	BUN	19	0.99	0.16	11.65	27.89	7.37	14.51	6.867	22.24	38.723	9.66	6.55
Cosby Creek	COS	15	1.04	0.00	8.39	49.99	11.85	36.45	5.52	8.98	17.25	3.21	4.84
Flat Creek	FLT	17	0.96	7.69	21.39	37.97	8.79	9.97	16.27	29.87	38.50	6.96	3.31
Hazel Creek	HAZ	12	0.95	3.47	9.99	23.18	5.80	16.74	5.09	12.56	23.00	5.78	7.57
Indian Camp Creek	ICC	15	0.99	1.02	4.80	9.72	2.71	13.94	7.33	10.92	14.89	2.54	4.21
Lost Bottom Creek	LOB	16	0.99	1.64	8.02	14.88	4.62	14.41	1.99	4.91	7.72	1.51	2.93
Road Prong	RPR	18	0.97	0.10	2.33	6.99	1.75	17.66	1.80	5.64	10.71	2.21	6.58
Rock Creek	ROC	20	1.01	0.00	2.59	11.26	3.20	27.62	0.76	4.14	7.61	2.11	7.01
Sams Creek	SAM	14	0.95	0.15	3.72	11.70	3.09	22.22	1.79	10.76	19.83	4.50	8.39
Silers	SIL	14	0.92	0.21	3.10	6.45	2.15	18.54	3.51	5.91	8.50	1.72	5.08
Mean		**15**	**0.97**	**1.82**	**7.75**	**19.51**	**5.09**	**20.12**	**5.13**	**11.22**	**17.84**	**3.84**	**5.78**

20.9%. The RSE among sites (spatial) was greater than the RSE within sites (temporal) for all adult brook trout populations and 73% of the YOY populations.

Hydrology, basin, and stream chemistry factors influenced the presence or absence of brook trout in GRSM streams as demonstrated by Tukey HSD tests between allopatric brook trout (n=11) and fishless sites (n=5). Significant models ($\alpha = 0.05$) are reported in Table 10. Higher concentrations of ANC, sodium and pH, and lower concentrations of sulfate and anions were associated with the presence of brook trout. Comparison tests also indicated that brook trout would more likely be present in streams with lower median flows (μ=0.12 cms, SD=0.06) in smaller basins (μ=4.24 km^2, SD=2.38 km^2) than in fishless streams with 0.19 ± 0.07 cms median flows in 7.55 ± 3.19 km^2 basin areas. The presence of brook trout in these streams was also associated with higher normalized fall flows (September, October, and November) and lower normalized January and February flows. Additionally, brook trout were more likely to be present in streams with higher

Table 10: Significant Tukey-Kramer HSD comparisons of hydrology, basin and chemistry variables between allopatric brook trout sites (n=11) and fishless sites (n=5).

	Independent Variable	Direction	P Value
Hydrology Factors	Median Q	–	0.0330
	January Q	–	0.0198
	February Q	–	0.0091
	September Q	+	0.0034
	October Q	+	0.0001
	November Q	+	0.0072
Basin Factors	Basin Area	–	0.0354
	Soil CEC	+	0.0174
	Soil Ksat	–	0.0196
Chemistry Factors	AVG pH	+	<0.0001
	AVG ANC	+	0.0008
	AVG SO_4	–	0.0095
	AVG Na	+	0.0018
	AVG ANION	–	0.0137

area-weighted average soil CEC concentrations and lower soil hydraulic conductivities in their respective basins.

Adult brook trout densities were positively correlated with elevation (ρ=0.618, p = 0.042), soil CEC concentration (ρ=0.673, p = 0.023), and percentage of high elevation mesic forests (ρ=0.773, p = 0.005), but were not significantly correlated with chemistry or hydrology variables. YOY trout densities were positively correlated with average pH (ρ=0.627, p = 0.038), soil pH (ρ=0.700, p = 0.017), soil depth (ρ=0.655, p = 0.029), and high elevation mesic forests (ρ=0.618, p = 0.043); and negatively correlated with average sulfate concentration (ρ=-0.718, p = 0.013). Total brook trout densities were additionally positively correlated with sodium (ρ=0.655, p = 0.029) and chloride (ρ=0.618, p = 0.043) concentrations.

Brook trout densities were significantly higher in streams with average sulfate concentrations less than 20 μeq/L (Figure 15.b) and average sodium concentration greater than 34 μeq/L (Figure 15.c). Condition factor, K, values were positively correlated with nitrate concentrations (ρ=0.655, p = 0.029) and negatively correlated with reversals (ρ=-0.782, p = 0.005) and percentage of Anakeesta in stream basins (ρ=-0.663, p = 0.026). Lower variability within YOY brook trout populations (RSE) was associated with higher November flows (ρ=-0.700, p = 0.017), basin elevations (ρ=-0.845, p = 0.001), and soil CEC concentrations (ρ=-0.673, p = 0.023). Figure 15.a illustrates the linear regression of YOY brook trout variability with soil CEC concentration (YOY RSE = 0.55-0.07(Soil CEC), n=11, p=0.0084, r^2=0.51). Higher May flows and steeper average soil slopes were associated with higher variability in YOY populations (ρ=0.837, p = 0.001; ρ=0.700, p = 0.017).

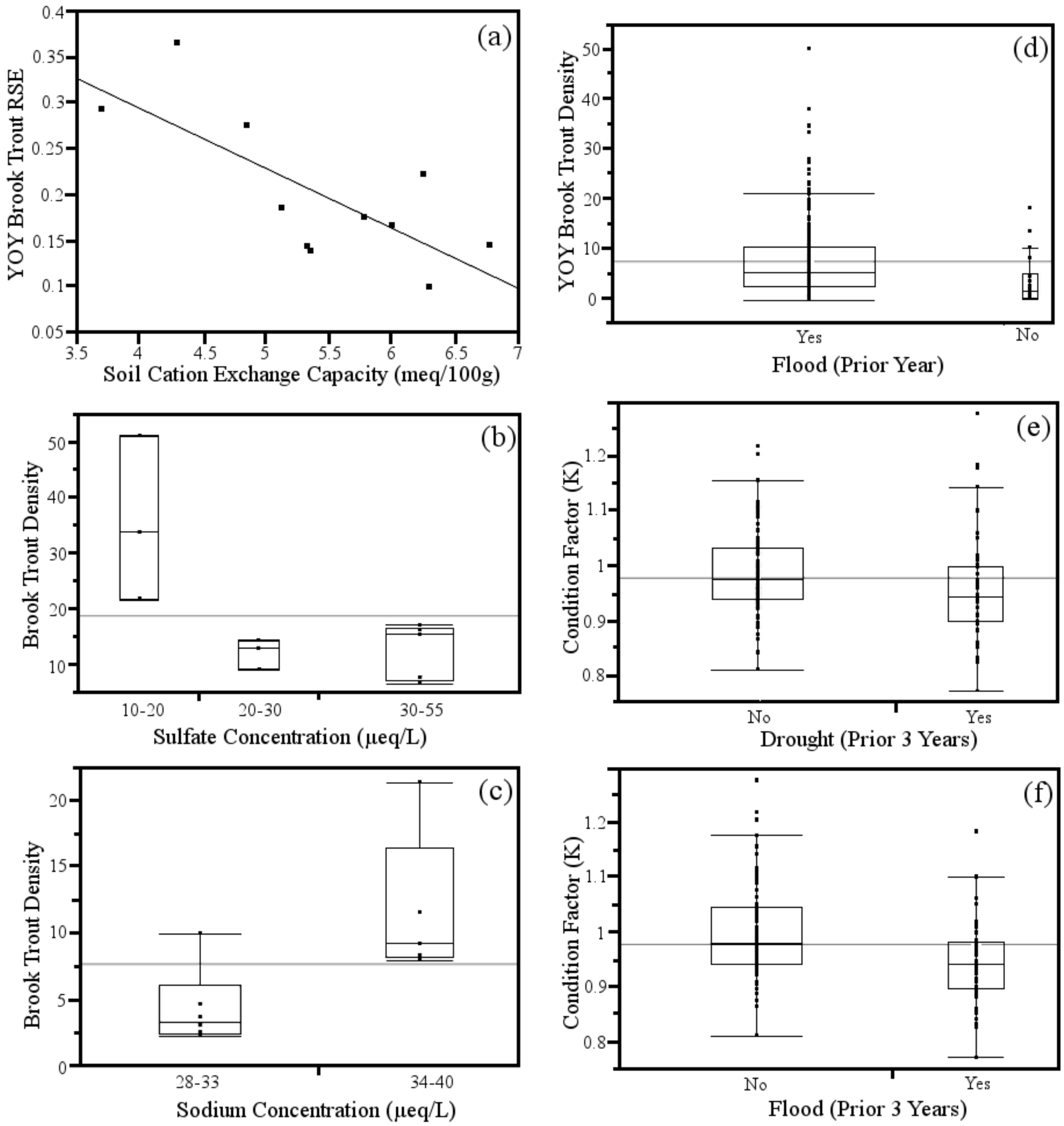

Figure 15: (a) Simple linear regressions of YOY brook trout RSE by soil CEC concentration: YOY RSE = 0.55-0.07(Soil CEC); (p=0.0084. r^2=0.51). (b) Tukey-Kramer HSD comparison of brook trout densities between ranges of sulfate concentrations. Densities in streams with sulfate concentrations between 10-20 µeq/L were significantly higher ($p<0.05$) than higher sulfate class ranges. (c) Significant pairwise difference ($p<0.05$) in trout densities between streams of different sodium concentration classes. (d) Significant differences ($p<0.05$) in YOY trout densities between occurrence of flood within one year of sampling date. (e) Significant comparison ($p<0.05$) in condition factor, K, between occurrence of drought within 3 years of sampling date. (f) Significant comparison ($p<0.05$) in condition factor, K, between occurrence of flood within 3 years of sampling date.

Tukey-Kramer HSD tests of brook trout densities among hydrological categorical data provided evidence that hydrologic factors potentially impact brook trout populations (Figures 15.d-15.f). YOY trout densities were significantly lower when there was a flood within one year of the sampling date (Figure 15.d), but not significantly lower when floods occurred within three years of sampling. Adult trout densities were not significantly different following hydrologic disturbances (flood or droughts). Condition factor, K, values of brook trout were significantly lower when drought occurred within three years before sampling date (Figure 15.e), and when floods occurred within one year and three years of fish sampling (Figure 15.f). When fish sampling occurred during a drought, adult and YOY brook trout densities were significantly higher.

Significant linear relationships were found between brook trout densities and chemical, hydrologic, and basin variables in univariate regression models. Two models that explained the greatest proportion of variability (highest r^2) in each of these variable groupings are reported in Table 11. Although significant regression models involving brook trout condition factors and independent variables were constructed, models explained less than 9% of variability in K values. Biotic relationships were found between adult and YOY brook trout densities and the previous year's sample. Adult and YOY brook trout densities from the previous year explained 69% and 44% of the variance in adult brook trout density. In YOY brook trout regressions, adult and YOY brook trout densities from the prior year explained 39% and 25% of the variance respectively.

Basin factors accounted for the greatest proportion of variability of the chemical, hydrological, and basin factor groups with respect to YOY and adult brook trout densities. Linear models of YOY brook trout densities versus area-weighted soil organic percentage

Table 11: Simple and multiple linear regressions of adult and YOY brook trout densities by chemical, hydrological, biotic, and basin variables. For simple linear regression models, the two variables that explained the greatest proportion of variability (highest r^2) in each of the factor groupings are reported. Final multiple regression models did not include biotic factors; reported models had the fewest number of regressors that explained the greatest amount of variability. Direction refers to the sign of the coefficient each regressor.

	Young of Year Brook Trout Densities				Adult Brook Trout Densities			
	Independent Variable	**Direction**	**N**	**R^2**	**Independent Variable**	**Direction**	**N**	**R^2**
Hydrology Factors	Median Q (prior WY)	–	148	0.13	Min Q (S,O,N)	–	148	0.16
	Max Q (M,A,M; prior Y)	–	148	0.09	Median Q (prior WY)	–	148	0.14
Basin Factors	Soil Organic %	+	162	0.44	Soil Slope	–	162	0.60
	Soil Slope	–	162	0.45	Site Elevation	+	162	0.55
Chemistry Factors	AVG Na	+	162	0.42	AVG Cl	+	162	0.33
	AVG SO_4	–	162	0.31	AVG Na	+	162	0.37
Biotic Factors	Adult BKT (prior year)	+	151	0.39	Adult BKT (prior year)	+	151	0.69
	YOY BKT (prior year)	+	151	0.25	YOY BKT (prior year)	+	151	0.44
Combined Models				**Prob>|t|**				**Prob>|t|**
	Median Q (prior WY)	–	110	<0.0001	Reversals	–	118	0.0005
	Soil Organic %	–	110	<0.0001	Soil Organic %	–	118	<0.0001
	AVG pH	+	110	<0.0001	AVG Cl	+	118	<0.0001
	1YR SO_4	+	110	<0.0001	AVG K	+	118	<0.0001
	1 YR Mg	–	110	0.0004	1 YR Ca		118	0.0005
		Adjusted R^2 = 0.69				**Adjusted R^2** = 0.74		

and average soil slope had r^2 values of 0.44 and 0.45 respectively (Table 11). In adult brook trout regressions, soil slope and site elevation explained 60% and 55% of the variance. Slopes of the regression models suggest that in basins with steeper slopes and soil slopes, trout densities were lower; and in streams at higher elevations, trout densities were higher. Other positively associated significant basin regressors of adult trout densities included soil CEC, soil organic, soil depth, and soil pH; other significant negatively associated regressors included basin area, channel slope, Anakeesta area, and mean basin slope (Table 11).

Of the three factor groups, chemical variables explained the second most variability in trout densities (Table 11). Sodium was the strongest water chemistry YOY trout density regressor ($r^2 = 0.42$) and adult trout density regressor ($r^2 = 0.37$). Higher concentrations of sodium and chloride were associated with higher brook trout densities whereas higher concentrations of sulfate were associated with lower densities. A significant regression between mean pH and YOY trout density suggested higher YOY densities in higher pH waters. Hydrology regressors explained the least amount of variance in the regression models (Table 11).

Significant ($p<0.05$) multiple regression models (produced using stepwise regression) to predict adult and YOY brook trout densities from hydrological, basin and chemical predictive variables, explained the variance in the trout density data to a greater extent than simple linear regressions (Table 11). The YOY trout density model had an r^2 value of 0.69 ($p<0.0001$) and included regressors median flow the preceding water year, soil organic percentage, mean stream pH, and average magnesium and sulfate concentrations the year preceding trout sampling. The adult trout density model had an r^2

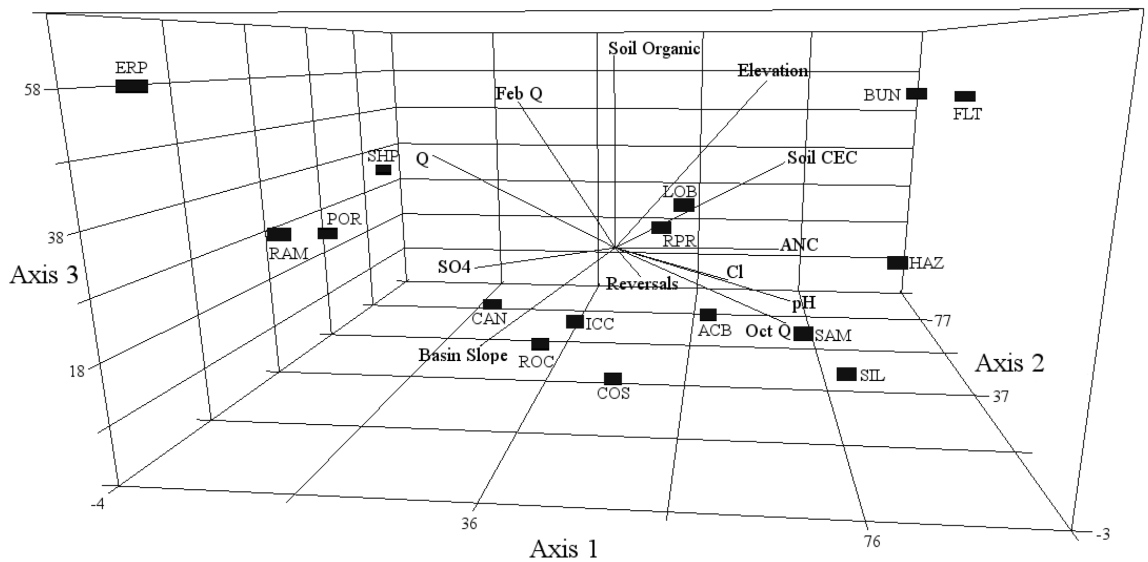

Figure 16: Loading plot and score plot of PCA of chemical, hydrological and basin variables in 16 collocated sites considered suitable for brook trout populations based on basin area. Variables included from each factor class: hydrology (median Q, reversals, September Q, March Q); chemistry (pH, Na, Cl, Al); and basin (soil slope, Anakeesta area, site elevation, and soil CEC). Site abbreviation for the 12 sites with brook trout population can be found in Table 9. Sites without brook trout include Shutts (SHP), Cannon (CAN), Eagle Rocks (ERP), Porters (POR) and Ramsey (RAM).

value of 0.74 ($p<0.0001$) and included regressors hydrologic reversals, soil organic percentage, mean concentration of calcium the year preceding trout sampling, and average chloride and potassium concentrations.

The PCA analyses illuminated relationships among study sites and demonstrated environmental factor loadings defining these relations (Figure 16). The first three principal components explained 80.9% (48.6%, 18.0%, 14.4%) of the variance in total brook trout densities. Component 1 of the PCA segregated all of the fishless sites from stream sites with brook trout populations. Primary negative factors of component 1 included sulfate concentrations, mean slope, median flows, and normalized February flows. Primary positive factors of component 1 include pH, ANC and chloride concentrations, elevation, soil CEC, and normalized October flows. Fishless sites (Shutts Prong, Eagle Rocks Prong, Ramsey Prong, Cannon Creek, and Porters Creek) were associated with higher values of the negative factors and lower values of the positive factors than brook trout sites.

The PCA demonstrated three distinguishable groups: 1) the two sites with the greatest densities of brook trout (Flat Creek and Bunches Creek), 2) the other nine brook trout sites and the fishless Cannon Creek, and 3) the other four fishless sites. Flat Creek and Bunches Creek, the two stream sites with the greatest trout densities, were separated from the other brook trout sites primarily from component three and to lesser extent components one and two. Primary factors of component 2 include soil organic percentage and chloride concentration (positive eigenvectors), and hydrologic reversals and median flow (negative eigenvectors). Primary positive factors of component 1 include pH, ANC and chloride concentrations, elevation, soil CEC, and normalized October flows.

4. Discussion

Chemical, hydrological, and basin factors influenced the presence or absence of brook trout in GRSM streams. Brook trout occupied streams with higher pH, ANC, and sodium concentrations, as in Appalachian streams reported elsewhere (Baldigo et al., 2007; Van Sickle et al., 1996). Soil cation exchange capacity, which influences baseflow and stormflow chemistry in the GRSM by providing more base cations and ANC concentrations to stream water, influenced the presence of brook trout such that in basins with higher CEC concentrations, brook trout were more likely to be present. Brook trout populations were more likely to be found in streams with higher median monthly flows in autumn months when brook trout spawn (Etnier and Starnes, 1993). This may indicate available spawning habitat is a limiting condition of brook trout distribution (Hakala and Hartman, 2004).

Interestingly, trout densities were higher in streams with higher sodium concentrations. This indicates that, at the population level, sodium may ameliorate the effects of acid toxicity, including sublethal effects resulting in downstream immigration (Gagen et al., 1994) and less successful reproduction (Kaeser and Sharpe, 2001). Toxic metal ions including monomeric inorganic aluminum and proton compete with critical metal ions on channel proteins in the gill surface (Di Toro et al., 2001) and facilitate ion regulatory failure from loss of critical blood ions including sodium and chloride (Wood et al., 1990). Increased ion permeability from acid toxicity has been shown to be ameliorated in trout by calcium (McDonald, 1983; Playle et al., 1989) and sodium (Abdul-Latif, 2008; Brown, 1981; Dietrich et al., 1989). Although trout toxicity was not examined in this research, this study provides evidence that elevated sodium concentrations may ameliorate the impacts of acid toxicity, and enable trout populations to be more successful in GRSM streams.

In addition to stream chemistry, densities of brook trout were also influenced by basin factors. Basin factors accounted for the greatest proportion of variability in brook trout densities, suggesting that successful trout populations are regulated by physical basin factors which govern the chemical, hydrologic, and habitat environment (Kocovsky and Carline, 2005; Richter et al., 1997). The best multiple regression models included a combination of chemical, hydrological, and basin factors, indicating the interaction of these factors influence brook trout density to a greater degree than independent factors.

The temporal variability in YOY brook trout populations was more than twice as great as the variability in adult populations in streams with viable trout populations. This suggests that YOY trout may be more susceptible to hydrologic disturbances and episodes of stream acidification as described in the literature (Baldigo and Lawrence, 2001; Carline and McCullough, 2003). YOY brook trout densities were significantly lower after large flood events; however, adult densities were not significantly different after these events. This is likely because adult fish have stronger swimming abilities and can find refugia in complex habitat morphology, and incubating eggs and YOY trout may be swept downstream in strong currents (Harvey, 1987). Surprisingly, channel slope and drainage area were not significantly correlated with the RSE of YOY trout. Although smaller, steeper streams have been found to have steeper shifts in brook trout populations due to increased frequency of extreme hydrological events (Elwood and Waters, 1969; Roghair et al., 2002), complex stream morphology and heavily forested riparian areas in the GRSM may provide adequate refugia for YOY fish from all but the most extreme hydrologic disturbances.

Abiotic and biotic factors influenced the condition factor, K, values of brook trout. Condition factor values were significantly lower after extreme flood events occurring within three years of sampling date. This may indicate floods reduced macroinvertebrate richness and biomass, a major food source of trout (Robinson and Uehlinger, 2008), or the mass of individual trout were reduced from energy consumption of returning upstream after flood displacement to occupy headwater reaches without food competition from rainbow trout in lower watershed reaches (Larson and Moore, 1985). The condition factor was higher when there was less temporal variation in adult trout density and when streams had higher cation concentrations. This may suggest that in streams buffered from hydrologic disturbances and acid episodes, fish are healthier; and in waters of lower ionic strength, sublethal effects from stream acidification impact trout health (McDonald et al., 1989). A positively sloped linear regression of k-factor versus time (Figure 21), significant at the $\alpha=0.05$ level, indicates fish health was improving during the 20 year period. This may be associated with non-significant increases in pH and ANC concentrations during this time period (Robinson et al., 2008), and could be one of the first indications that stream water quality is improving from reductions of acid deposition (NADP, 2009).

The GRSM fisheries management program is committed to protecting and restoring the native southern strain of brook trout in GRSM streams. This research, identifying abiotic and biotic factors that determine the distribution and density of brook trout, provides invaluable information such that the program can concentrate conservation or restoration efforts.

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Chapter VI: Summary

The primary focus of this research was to investigate the impacts of acid deposition on GRSM stream water quality and native southern brook trout to support the management of aquatic resources. In this investigation, relationships were developed between baseflow and stormflow chemical constituents; the effects of elevation, area, geology, soil, and vegetation on stream chemistry were examined; physiological distress in brook trout in episodes of stream acidification was evaluated; and the influence of stream chemistry, basin characteristics, and ecologically relevant hydrologic parameters on brook trout distribution, densities, and health was explored (Figure 17).

The first study (Chapter III) in this research examined the relationship between baseflow and stormflow and the effects of basin factors on stream chemistry. The label ***A*** in Figure 17 depicts the interactions investigated in this chapter. Strong linear relationships were exemplified between baseflow and stormflow pH and ANC concentrations in regression models. Following precipitation events, stream pH was reduced and aluminum concentrations increased, while the response of ANC, nitrate, sulfate, and base cations varied. Streams at higher elevations (>975 m) had significantly lower pH, ANC, sodium, and silicon and higher nitrate concentrations ($p<0.05$). Smaller streams (< 10 km^2) had significantly lower nitrate, sodium, magnesium, silicon, and base cation concentrations ($p<0.05$). In stormflow, streams in basins with Anakeesta geology (>10%) had significantly lower pH and sodium concentrations, and higher aluminum concentrations. Weight-averaged soil parameters and percentage of forest types in basins additionally contributed to unique stream acidification response. Several basin characteristics were highly correlated demonstrating the interrelatedness of topographic, geologic, soil, and

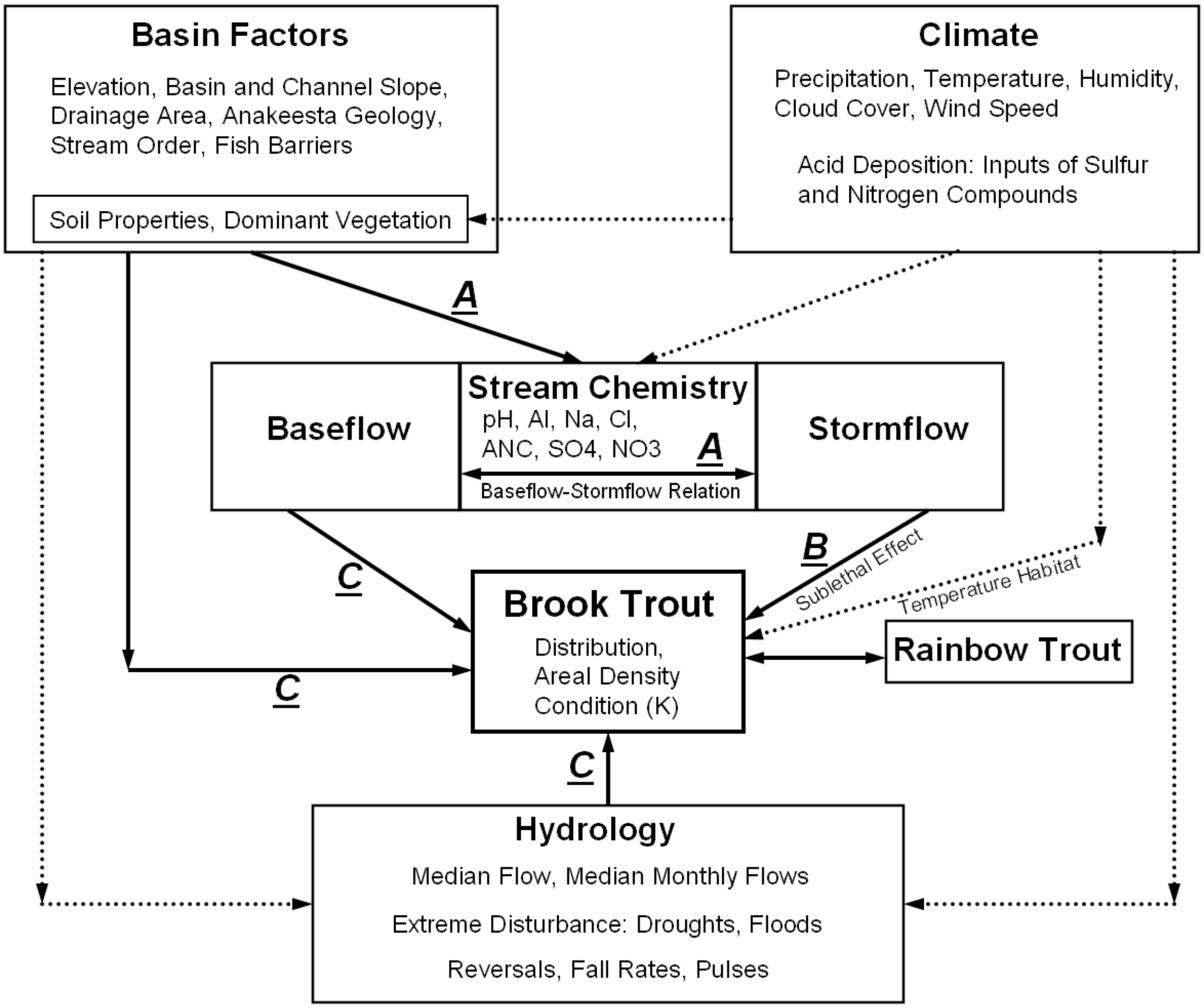

Figure 17: Conceptual model of influences on brook trout distribution, densities, and condition factor, K, values. Solid lines indicate interactions examined in this research. Dashed lines indicate other interactions not examined. Letters designate which study investigated the relationship: *A* – basin factors influencing stream chemistry, *B* – in situ bioassays, *C* – influence of chemical, hydrological, and basin factors on brook trout.

vegetative parameters; these included elevation, drainage area, basin slope, chemical and physical soil characteristics, and percentage of forest types. These interrelated factors influenced baseflow and stormflow chemistry.

The second study (Chapter IV) in this research evaluated the physiological distress in brook trout in episodes of stream acidification. The label ***B*** in Figure 17 depicts the interaction investigated in this chapter. Brook trout were exposed to two acid episodes during *in situ* bioassays conducted in three GRSM streams. Stream pH decreased (> 0.7 pH units) and total dissolved aluminum (Al_{TD}) increased (> 0.175 mg/L) at all three sites during acid episodes in both bioassays. Whole-body sodium concentrations were significantly reduced (10-20%) following the acid episodes when preceding 24-h mean pH values (4.88, 5.09, 4.87) and corresponding 24-h time weighted average Al_{TD} concentrations (210, 202, 202 μg/L) were observed. Lower whole-body sodium concentrations were correlated with elevated H^+ and Al_{TD} concentrations.

The third study (Chapter V) in this research examined the influence of chemical, hydrological, biotic, and basin factors on brook trout distribution, densities, and condition. The label ***C*** in Figure 17 depicts the interactions investigated in this chapter. Basin factors accounted for the greatest proportion of variability in young-of-year (YOY) and adult brook trout densities. Adult brook trout densities were positively correlated ($p<0.05$) with elevation and average soil cation exchange concentration. Spatial variability was greater than temporal variability in trout populations, and temporal variability in YOY populations was more than double the variability in adult populations. This suggests that YOY trout may be more susceptible to hydrologic disturbances or episodes of stream acidification. Higher concentrations of ANC, sodium and pH were associated with the presence of brook trout. Trout densities were higher in streams with higher concentrations of sodium, suggesting that, at the population level, sodium may ameliorate the effects of acid toxicity. Fall flows were positively correlated ($p<0.05$) with total brook trout densities and YOY trout densities were significantly lower when there was a flood within one year of the sampling date. The interaction of chemical, hydrological, and basin factors influence brook trout distributions and densities.

This research provides invaluable information to the GRSM fisheries management program for conservation and restoration efforts. Interrelated basin characteristics including elevation, drainage area, basin slope, chemical and physical soil characteristics, and percentage of forest types influence stream acidification in GRSM streams. Loss of sodium ions in native southern brook trout were consistent with physiological distress resulting from acid exposure reported in salmonids in other investigations. It is unclear whether acid episodes are responsible for extirpation of brook trout from headwater streams in the GRSM or simply contribute to sublethal effects including downstream migration and depressed reproduction. Although brook trout populations are believed to have primarily been impacted by stream acidification in the GRSM, the interaction of chemical, hydrological, basin factors, and biotic interactions influence trout distributions and densities.

The GRSM is the second largest national park in the Eastern United States and receives approximately 10 million visitors per year, making it the most visited national park. GRSM streams support a great number of fish species, amphibians, and benthic invertebrates; five streams in the GRSM have been designated as Outstanding National Resource Waters. As an International Biosphere Reserve, it is essential to promote and demonstrate a balanced relationship between humans and the biosphere in this exceptional park. I hope that research presented in this dissertation can help support the mission of the National Park Service (NPS) to preserve the unimpaired natural resources for the enjoyment, education and inspiration of this and future generations (National Park Service Organic Act, 16 U.S.C.1.) by providing valuable data and analyses to facilitate environmental management decisions.

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Appendices

Appendix A: Supplementary Table for Basin Effects on Stream Chemistry Study

Tukey-Kramer HSD Comparisons of Baseflow and Stormflow Chemistry Between Block Groups

Tables 12 and 13 show differences in water chemistry constituents in baseflow and stormflow stream chemistry between block groups: elevation (high and low), area (small and large), and Anakeesta geology (present and absent). Differences are tested by ANOVA means comparisons (Tukey-Kramer HSD).

Table 12: Tukey-Kramer bivariate means comparison of chemical constituents in baseflow between elevation classes (high and low), area classes (small and large), and Anakeesta geology (present and absent).

Baseflow	Elevation	High	Average	6.12	28.22	27.90	37.51	0.55	30.90	24.35	50.79	0.045	2.38	116.10	77.07	1.20
			SD	0.59	25.02	18.86	15.53	0.99	6.21	6.72	11.50	0.380	0.53	14.05	33.44	0.64
		Low	Average	6.44	40.58	14.63	37.20	0.41	33.51	20.98	50.52	0.036	2.58	115.00	63.62	0.84
			SD	0.30	21.97	11.17	9.29	0.96	5.88	5.36	9.31	0.030	0.46	17.93	20.23	0.34
		ANOVA	p-value	<0.0001	0.0019	<0.0001	0.8855	0.3898	0.0102	0.0011	0.8783	0.1100	0.0195	0.6782	0.0042	0.0910
	Area	< 10 km2	Average	6.33	31.15	17.98	37.92	0.39	30.51	20.53	49.56	0.041	2.39	110.44	67.82	0.87
			SD	0.26	16.40	12.53	10.69	0.65	5.26	0.72	11.48	0.032	0.44	14.03	22.57	0.21
		10 km2 – 20 km2	Average	6.21	36.83	24.73	36.85	0.57	33.62	24.70	51.65	0.041	2.56	120.17	73.13	1.19
			SD	0.64	29.55	19.65	14.61	1.20	6.58	0.68	9.44	0.036	0.55	16.30	33.04	0.74
		ANOVA	p-value	0.1727	0.1593	0.0158	0.6195	0.2770	0.0021	<0.0001	0.2295	0.8912	0.0378	0.0002	0.2638	0.1288
	Anakeesta	> 10%	Average	6.14	28.29	25.20	40.33	0.35	30.15	23.68	52.78	0.044	2.36	116.17	77.27	1.10
			SD	0.58	23.94	20.31	11.80	0.55	5.12	6.92	9.27	0.037	0.48	12.60	31.08	0.69
		None	Average	6.43	41.45	16.97	33.65	0.65	34.65	21.55	48.02	0.036	2.63	114.83	62.34	0.90
			SD	0.31	22.97	9.87	13.29	1.32	6.49	5.28	11.32	0.030	0.50	19.48	22.82	0.20
		ANOVA	p-value	0.0003	0.0010	0.0032	0.0016	0.6040	<0.0001	0.0420	0.0060	0.1502	0.0011	0.6171	0.0015	0.3452

Table 13: Tukey-Kramer bivariate means comparison of chemical constituents in stormflow between elevation classes (high and low), area classes (small and large), and Anakeesta geology (present and absent).

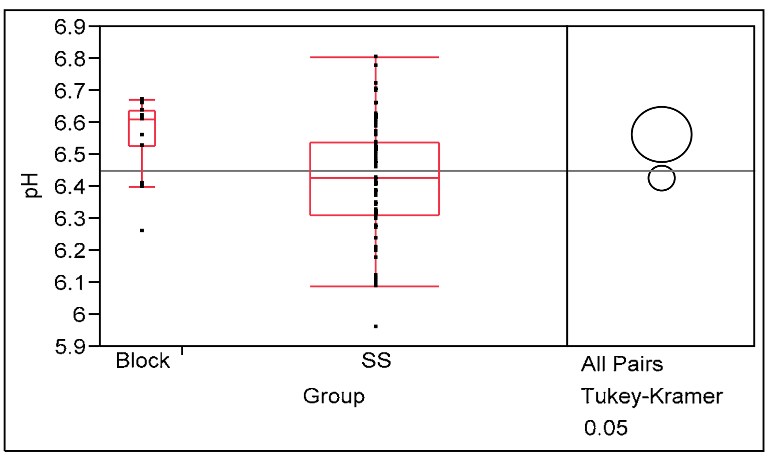
			pH	ANC	NO_3^-	SO_4^{2-}	NH_4^+	Na^+	Mg^{2+}	Ca^{2+}	Al	Si	BC	BA	DOC
Elevation	High	Average	5.80	26.15	21.89	40.12	0.71	25.10	24.52	56.73	0.149	1.76	120.79	73.61	3.47
		SD	0.71	30.20	16.84	18.67	1.15	7.03	6.23	11.34	0.124	0.52	15.84	35.37	1.37
	Low	Average	6.25	44.64	14.03	37.37	1.60	30.09	23.42	51.79	0.076	2.15	130.74	63.39	2.38
		SD	0.29	32.81	11.84	11.09	5.53	5.73	5.26	19.17	0.052	0.41	27.48	21.54	1.33
	ANOVA	p-value	<0.0001	0.0062	0.01	0.3888	0.3019	0.0003	0.3594	0.1316	0.0003	<0.0001	0.0384	0.0945	0.0359
Area	< 10 km2	Average	6.05	28.57	15.17	38.14	1.24	26.14	21.73	55.22	0.108	1.85	117.71	65.44	2.87
		SD	0.35	21.08	10.34	12.92	5.17	5.62	4.13	11.92	0.088	0.44	16.12	23.00	1.58
	10 km2 – 20 km2	Average	6.02	45.61	21.35	39.43	1.08	29.82	26.95	65.00	0.116	2.12	137.22	72.14	2.92
		SD	0.80	42.32	19.05	17.94	1.81	7.77	6.28	19.10	0.116	0.54	26.40	36.08	1.31
	ANOVA	p-value	0.7807	0.0129	0.0488	0.6883	0.8529	0.0098	<0.0001	0.0033	0.7080	0.0092	<0.0001	0.2804	0.9256
Anakeesta	> 10%	Average	5.88	33.36	19.48	41.02	0.88	25.48	24.48	63.04	0.154	1.89	126.67	71.83	3.28
		SD	0.74	38.35	17.82	15.09	1.58	0.93	6.29	17.92	0.121	0.56	24.17	31.97	1.34
	None	Average	6.20	38.45	15.95	36.14	1.50	30.13	23.36	55.36	0.064	2.04	125.28	64.39	2.50
		SD	0.26	25.50	10.78	15.01	5.68	0.97	5.07	12.70	0.032	0.42	22.12	25.82	1.46
	ANOVA	p-value	0.0071	0.4592	0.2592	0.1235	0.4656	0.00	0.3489	0.0209	<0.0001	0.1328	0.7668	0.2252	0.1348

Tukey-Kramer HSD Comparisons of Baseflow Chemistry Between Historic Stream Survey Sites and Block-Designed Sites

Tukey-Kramer HSD comparisons of baseflow stream chemistry between collocated historic stream survey sites and block-designed sites. In general, pH was significantly higher in block design sites, and ANC concentrations were not significantly different.

Lost Bottom Creek

Oneway Analysis of pH By Group



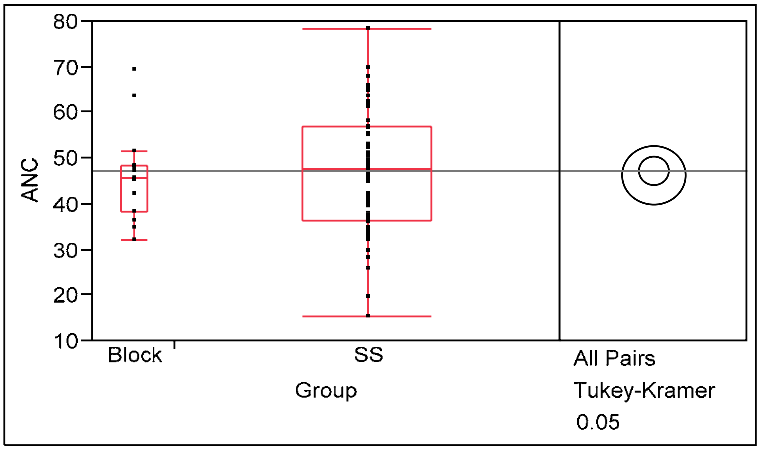
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
1.98861	0.05

Abs(Dif)-LSD	Block	SS
Block	-0.12211	0.041333
SS	0.041333	-0.05613

Positive values show pairs of means that are significantly different.

Oneway Analysis of ANC By Group



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

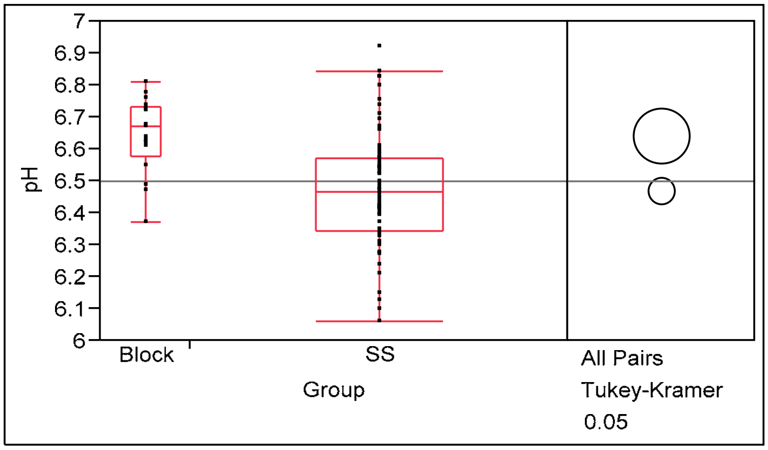
q*	Alpha
1.98861	0.05

Abs(Dif)-LSD	SS	Block
SS	-4.25618	-6.22218
Block	-6.22218	-9.25984

Positive values show pairs of means that are significantly different.

Palmer Creek

Oneway Analysis of pH By Group



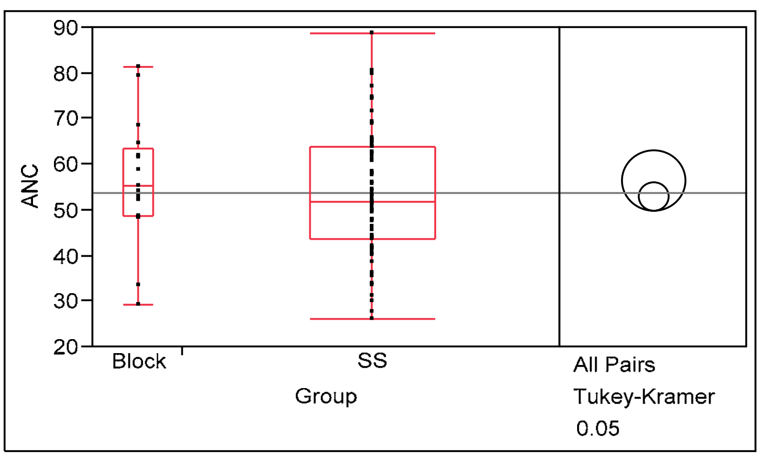
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
1.98761	0.05

Abs(Dif)-LSD	Block	SS
Block	-0.12066	0.081031
SS	0.081031	-0.05863

Positive values show pairs of means that are significantly different.

Oneway Analysis of ANC By Group



Means Comparisons

Comparisons for all pairs using Tukey-Kramer HSD

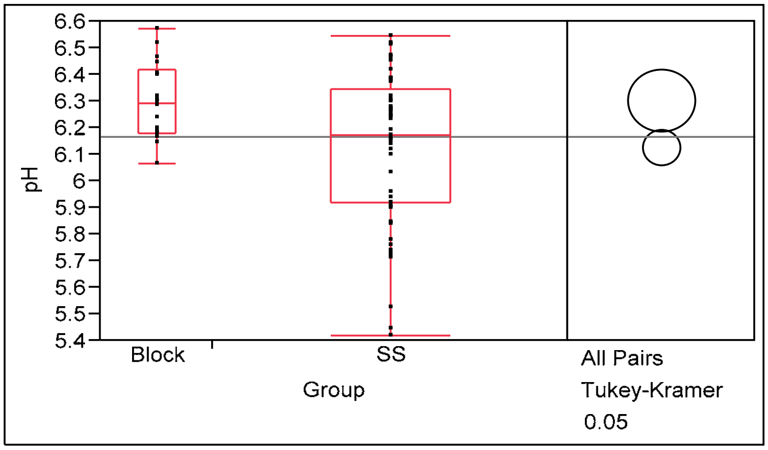
q*	Alpha
1.98793	0.05

Abs(Dif)-LSD	Block	SS
Block	-9.38116	-3.8819
SS	-3.8819	-4.59041

Positive values show pairs of means that are significantly different.

Road Prong

Oneway Analysis of pH By Group



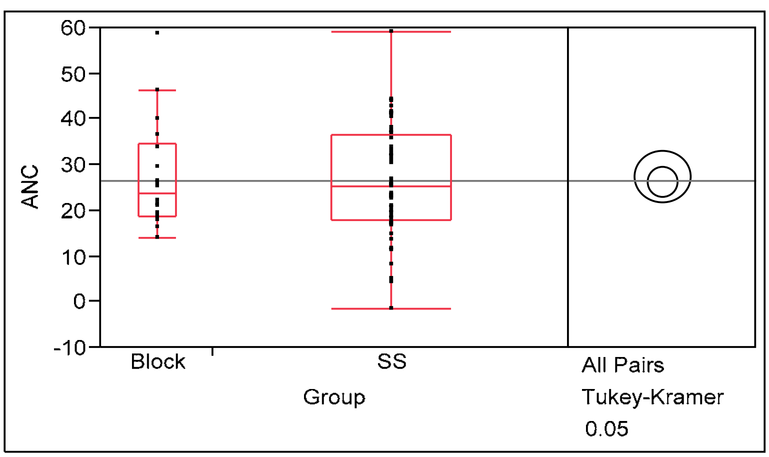
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
1.99308	0.05

Abs(Dif)-LSD	Block	SS
Block	-0.16644	0.039659
SS	0.039659	-0.09353

Positive values show pairs of means that are significantly different.

Oneway Analysis of ANC By Group



Comparisons for all pairs using Tukey-Kramer HSD

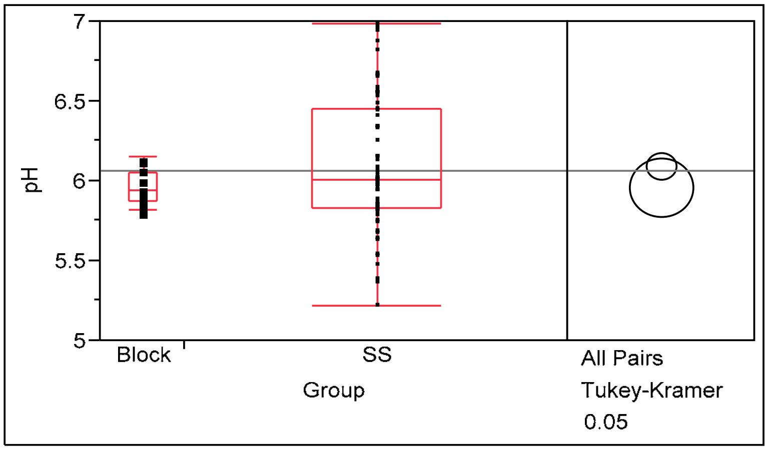
q*	Alpha
1.99308	0.05

Abs(Dif)-LSD	Block	SS
Block	-7.9794	-5.40075
SS	-5.40075	-4.48404

Positive values show pairs of means that are significantly different.

Rock Creek

Oneway Analysis of pH By Group



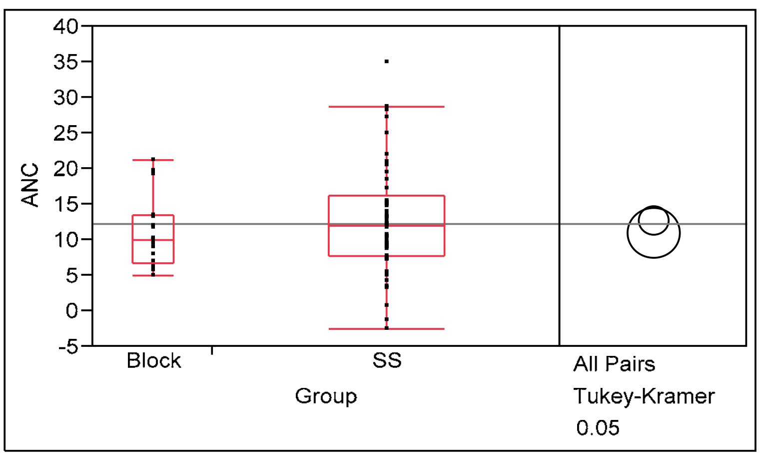
Comparisons for all pairs using Tukey-Kramer HSD

q*	Alpha
1.98609	0.05

Abs(Dif)-LSD	SS	Block
SS	-0.12238	-0.07017
Block	-0.07017	-0.26046

Positive values show pairs of means that are significantly different.

Oneway Analysis of ANC By Group



Comparisons for all pairs using Tukey-Kramer HSD

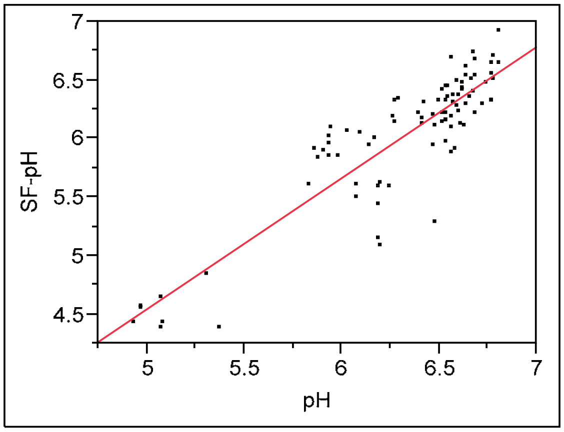
q*	Alpha
1.99780	0.05

Abs(Dif)-LSD	SS	Block
SS	-2.90641	-2.35262
Block	-2.35262	-4.93436

Positive values show pairs of means that are significantly different.

Chapter III Linear Regressions

Bivariate Fit of SF-pH By pH



Linear Fit

SF-pH = -1.045951 + 1.1193438*pH

Summary of Fit

RSquare	0.790981
RSquare Adj	0.788605
Root Mean Square Error	0.26881
Mean of Response	6.02761
Observations (or Sum Wgts)	90

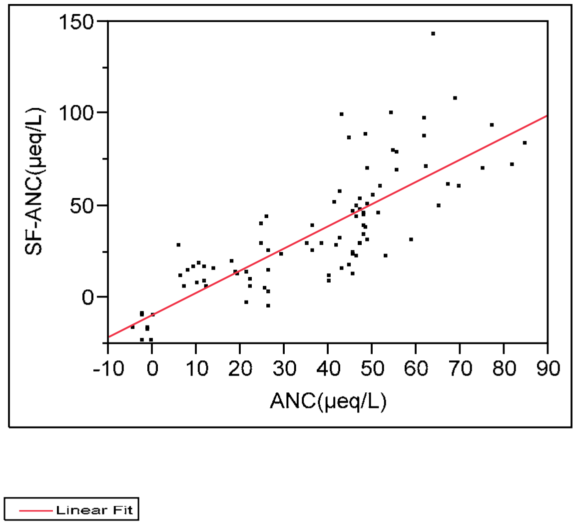
Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	1	24.063108	24.0631	333.0135
Error	88	6.358762	0.0723	**Prob > F**
C. Total	89	30.421870		<.0001*

Parameter Estimates

Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	-1.045951	0.388655	-2.69	0.0085*
pH	1.1193438	0.061338	18.25	<.0001*

Bivariate Fit of SF-ANC(µeq/L) By ANC(µeq/L)



Linear Fit

SF-ANC(µeq/L) = -8.859968 + 1.2073569*ANC(µeq/L)

Summary of Fit

RSquare	0.633302
RSquare Adj	0.629135
Root Mean Square Error	20.06912
Mean of Response	35.45271
Observations (or Sum Wgts)	90

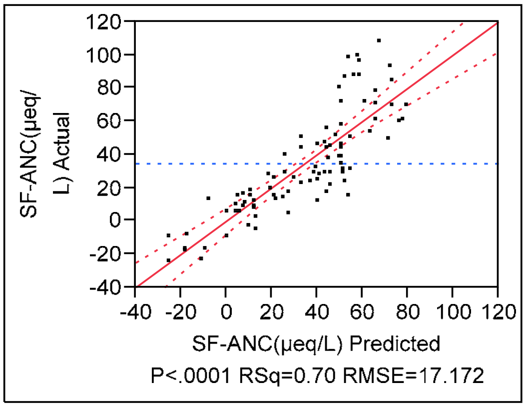
Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	1	61212.710	61212.7	151.9794
Error	88	35443.737	402.8	**Prob > F**
C. Total	89	96656.447		<.0001*

Parameter Estimates

Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	-8.859968	4.170789	-2.12	0.0365*
ANC(µeq/L)	1.2073569	0.097936	12.33	<.0001*

Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.704874
RSquare Adj	0.694458
Root Mean Square Error	17.1716
Mean of Response	34.24248
Observations (or Sum Wgts)	89

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	59861.153	19953.7	67.6709
Error	85	25063.434	294.9	**Prob > F**
C. Total	88	84924.588		<.0001*

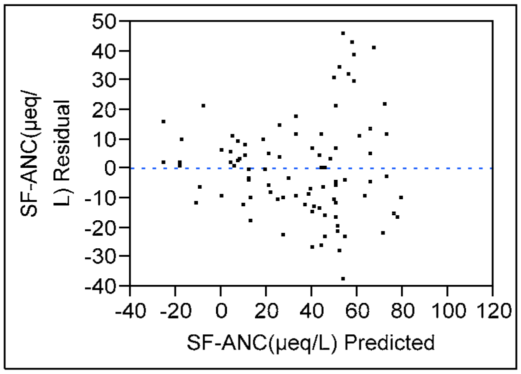
Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	68	20045.978	294.794	0.9988
Pure Error	17	5017.457	295.145	**Prob > F**
Total Error	85	25063.434		0.5319
				Max RSq
				0.9409

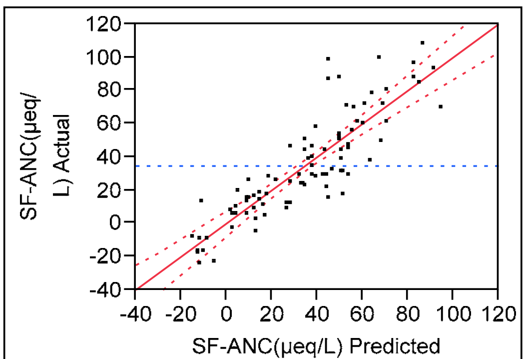
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	VIF
Intercept	-163.8236	34.80545	-4.71	<.0001*	.
pH	30.027585	5.435382	5.52	<.0001*	1.9140308
Cl(µeq/L)	-5.15351	1.464784	-3.52	0.0007*	1.0401395
Na(µeq/L)	2.162105	0.432635	5.00	<.0001*	1.8601209

Residual by Predicted Plot



Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.762263
RSquare Adj	0.750942
Root Mean Square Error	15.50336
Mean of Response	34.24248
Observations (or Sum Wgts)	89

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	64734.841	16183.7	67.3328
Error	84	20189.746	240.4	**Prob > F**
C. Total	88	84924.588		<.0001*

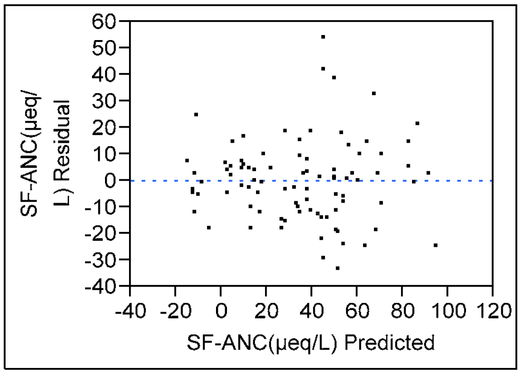
Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	67	15172.290	226.452	0.7673
Pure Error	17	5017.457	295.145	**Prob > F**
Total Error	84	20189.746		0.7820
				Max RSq
				0.9409

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	VIF
Intercept	133.46254	24.92048	5.36	<.0001*	.
Mean Slope	-4.734024	0.891562	-5.31	<.0001*	2.0750943
ANC(µeq/L)	0.7650689	0.102279	7.48	<.0001*	1.7959188
Cl(µeq/L)	-5.282034	1.398566	-3.78	0.0003*	1.1632688
Cation Sum	0.5264641	0.148755	3.54	0.0007*	1.8627534

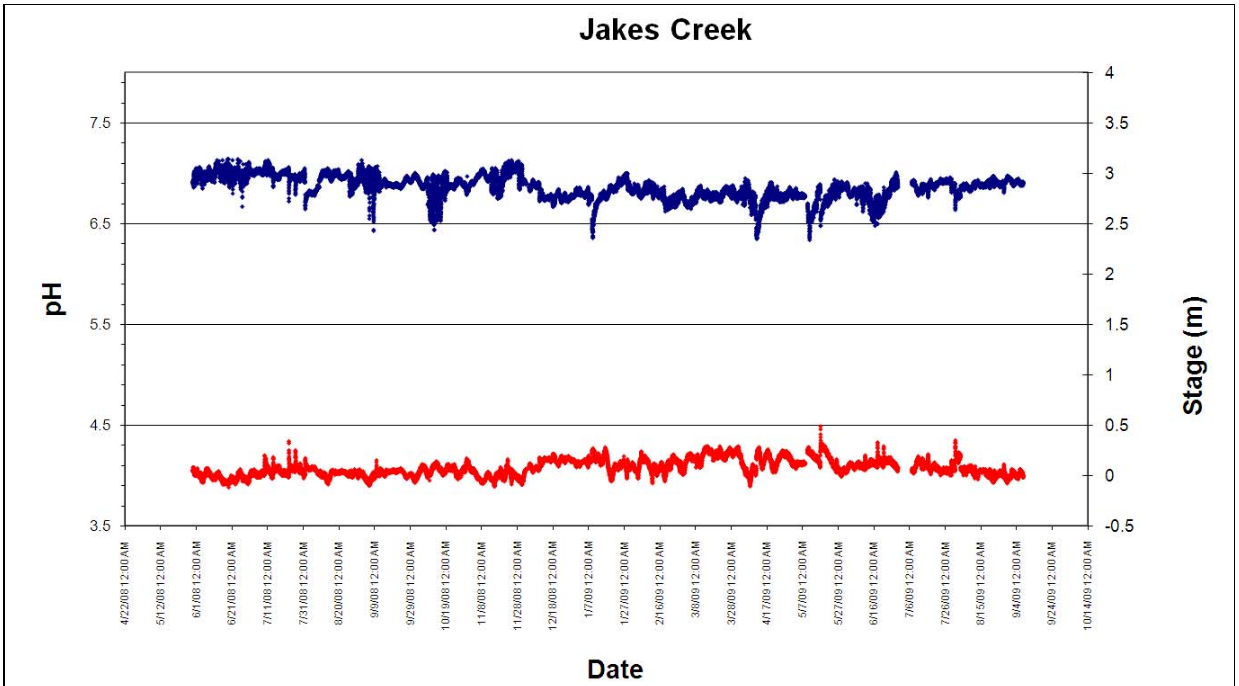
Residual by Predicted Plot

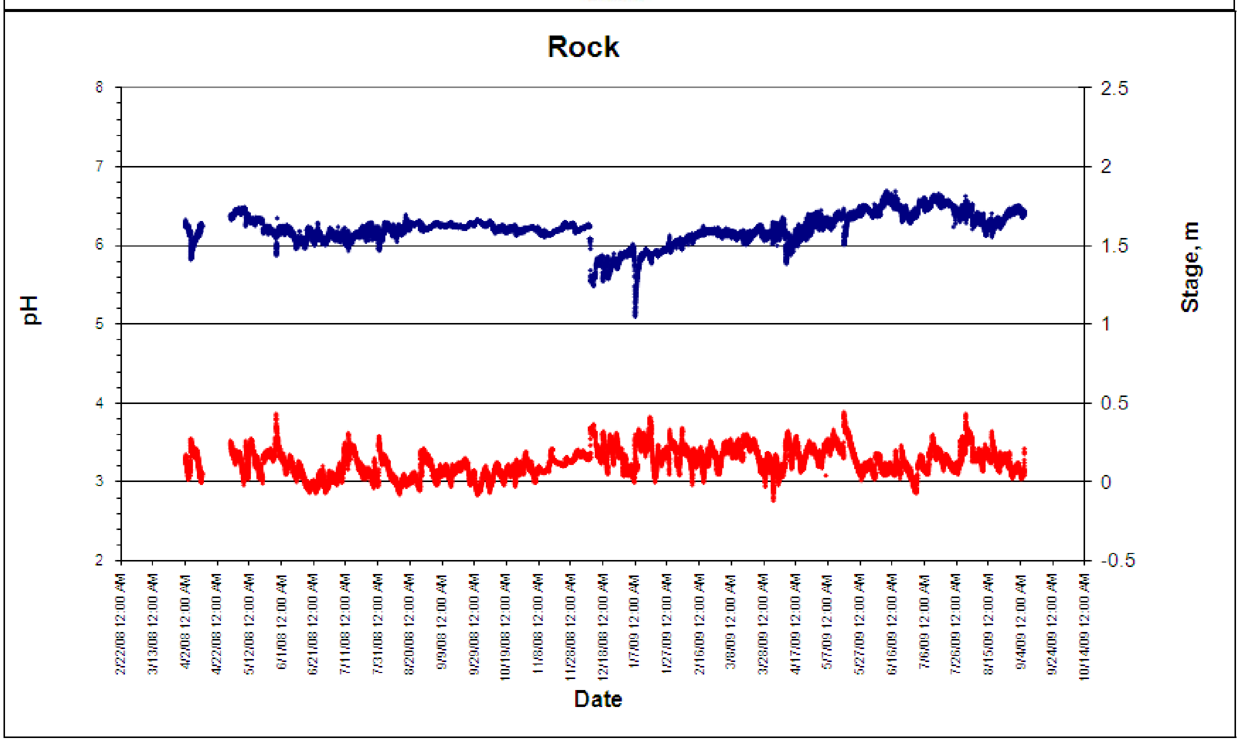


Sonde Data

Raw sonde data, illustrated in subsequent figures, was used by Mauney to develop pH CDF curves to characterize episodic acidification responses during stormflows for different streams.

Mauney III, John Leland, "Characterizing Episodic Stream Acidification Using a Concentration-Duration-Frequency Methodology in Watersheds of the Great Smoky Mountains National Park." Master's Thesis, University of Tennessee, 2009. http://trace.tennessee.edu/utk_gradthes/544


Jakes Creek
pH
Stage (m)
Date
7.5
6.5
5.5
4.5
3.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
4/22/08 12:00 AM
5/12/08 12:00 AM
6/1/08 12:00 AM
6/21/08 12:00 AM
7/11/08 12:00 AM
7/31/08 12:00 AM
8/20/08 12:00 AM
9/9/08 12:00 AM
9/29/08 12:00 AM
10/19/08 12:00 AM
11/8/08 12:00 AM
11/28/08 12:00 AM
12/18/08 12:00 AM
1/7/09 12:00 AM
1/27/09 12:00 AM
2/16/09 12:00 AM
3/8/09 12:00 AM
3/28/09 12:00 AM
4/17/09 12:00 AM
5/7/09 12:00 AM
5/27/09 12:00 AM
6/16/09 12:00 AM
7/6/09 12:00 AM
7/26/09 12:00 AM
8/15/09 12:00 AM
9/4/09 12:00 AM
9/24/09 12:00 AM
10/14/09 12:00 AM


Rock
pH
Stage, m
Date
8
7
6
5
4
3
2
2.5
2
1.5
1
0.5
0
-0.5
2/22/08 12:00 AM
3/13/08 12:00 AM
4/2/08 12:00 AM
4/22/08 12:00 AM
5/12/08 12:00 AM
6/1/08 12:00 AM
6/21/08 12:00 AM
7/11/08 12:00 AM
7/31/08 12:00 AM
8/20/08 12:00 AM
9/9/08 12:00 AM
9/29/08 12:00 AM
10/19/08 12:00 AM
11/8/08 12:00 AM
11/28/08 12:00 AM
12/18/08 12:00 AM
1/7/09 12:00 AM
1/27/09 12:00 AM
2/16/09 12:00 AM
3/8/09 12:00 AM
3/28/09 12:00 AM
4/17/09 12:00 AM
5/7/09 12:00 AM
5/27/09 12:00 AM
6/16/09 12:00 AM
7/6/09 12:00 AM
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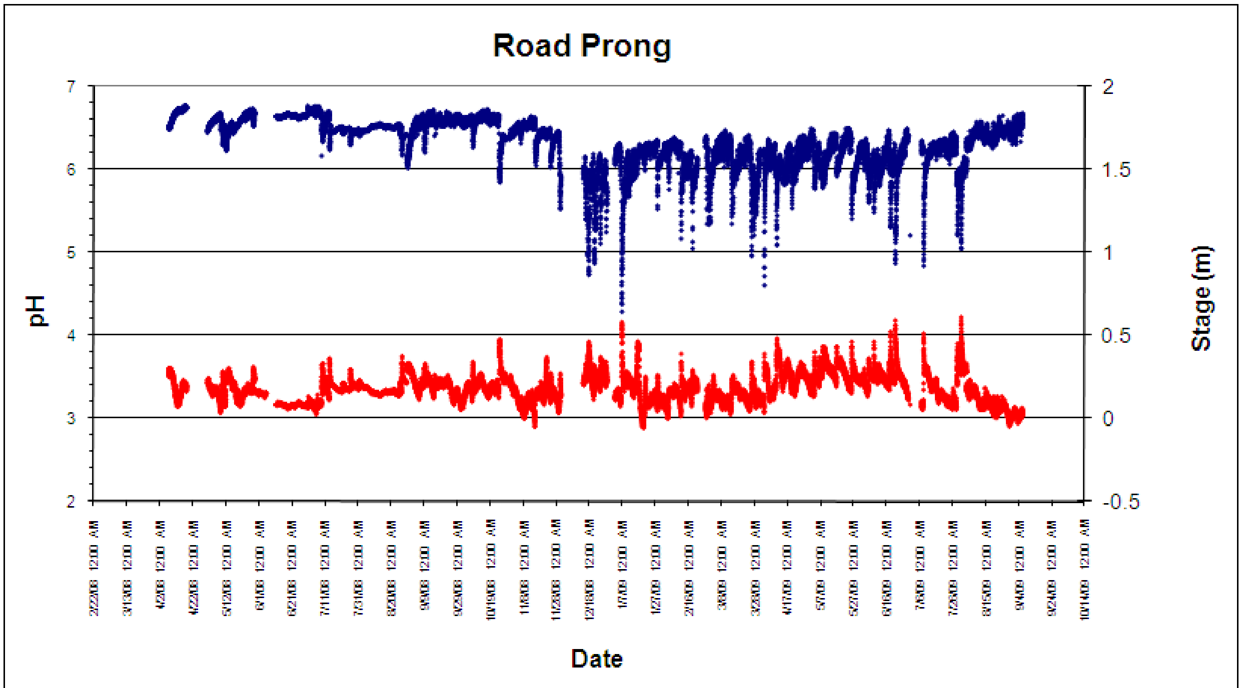
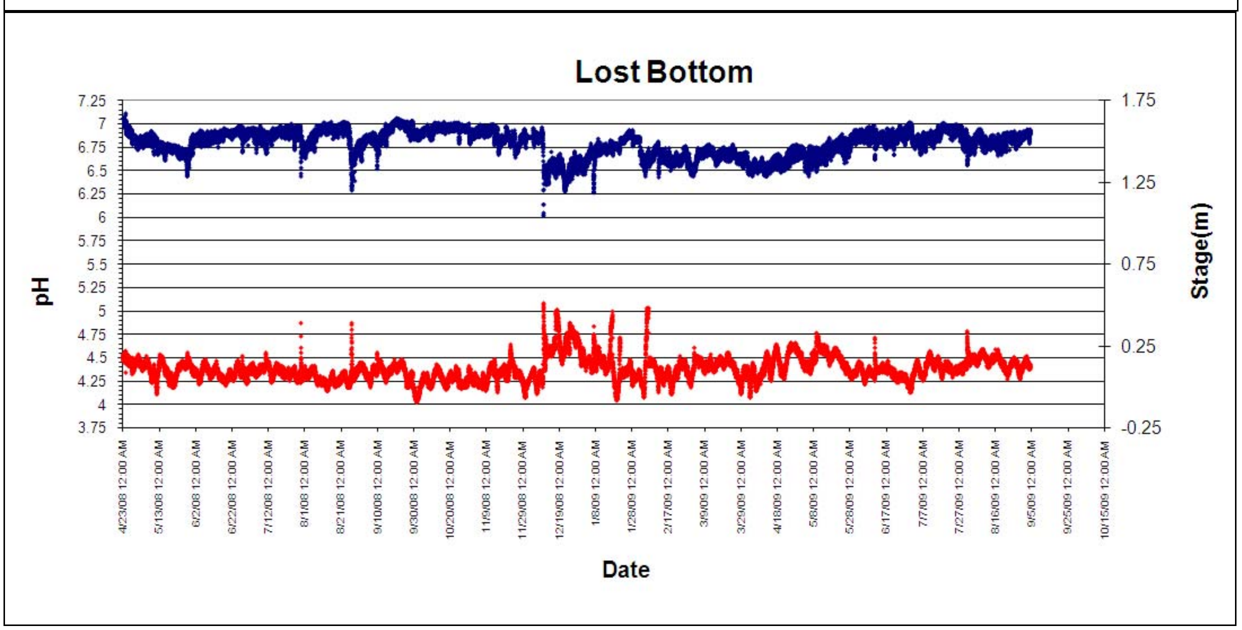
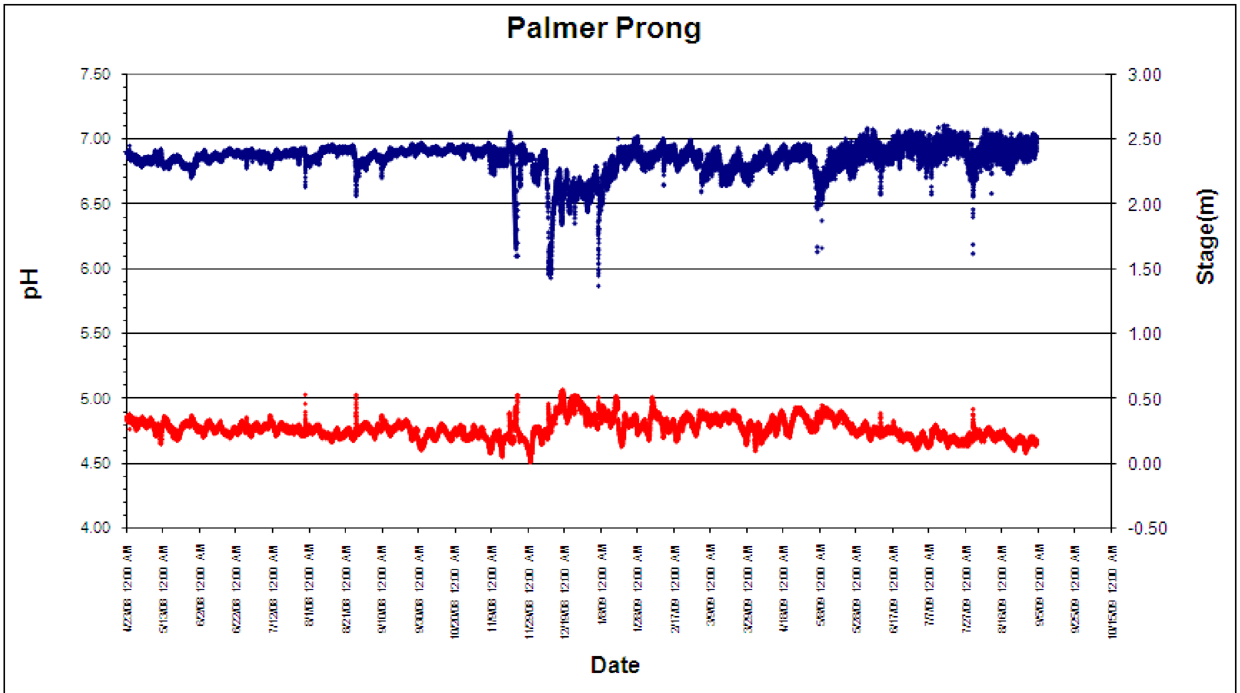
10/14/09 12:00 AM

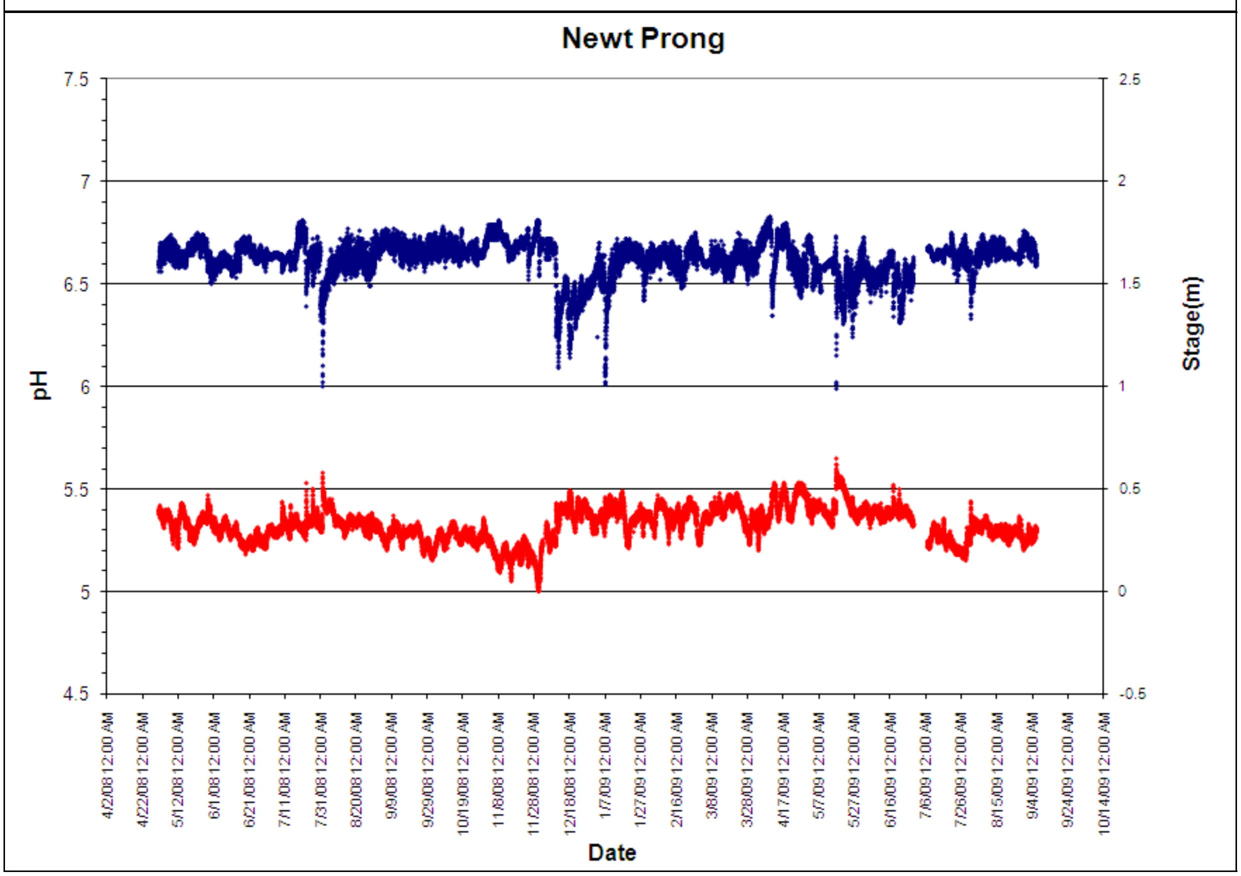


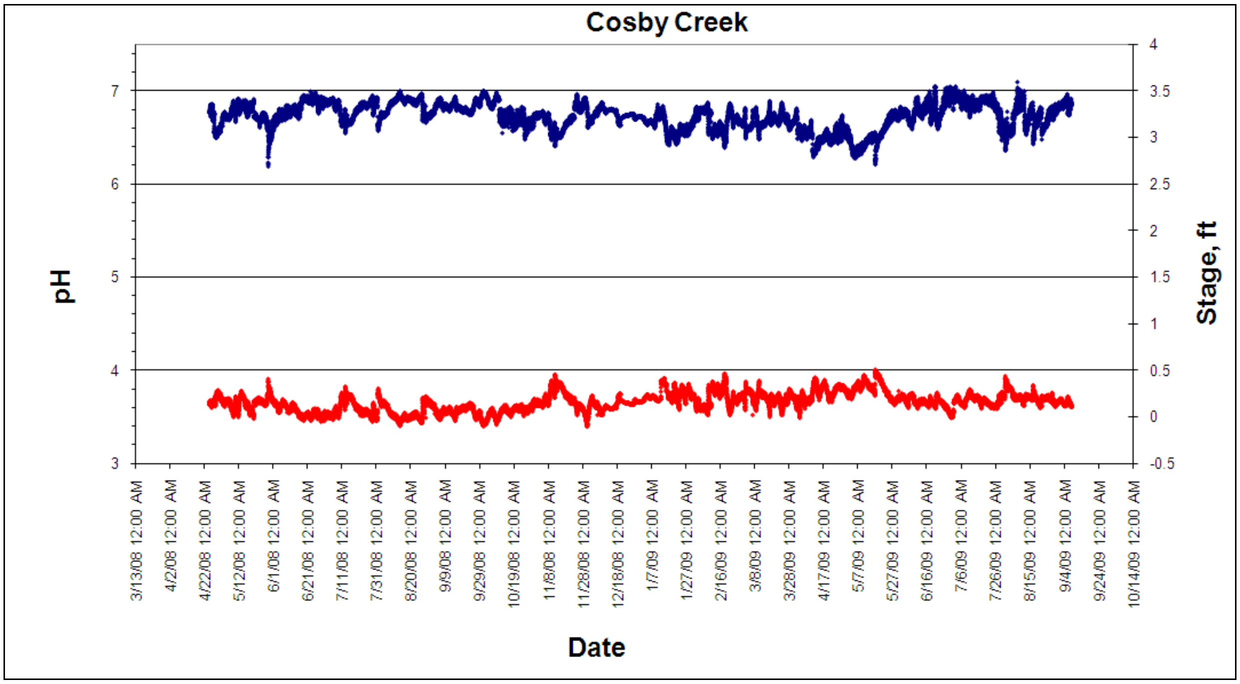
Road Prong
pH
Stage (m)
Date



Lost Bottom
pH
Stage(m)
Date


Palmer Prong
pH
Stage(m)
Date
7.50
7.00
6.50
6.00
5.50
5.00
4.50
4.00
3.00
2.50
2.00
1.50
1.00
0.50
0.00
-0.50


Newt Prong
pH
Stage(m)
Date
7.5
7
6.5
6
5.5
5
4.5
2.5
2
1.5
1
0.5
0
-0.5
4/2/08 12:00 AM
4/22/08 12:00 AM
5/12/08 12:00 AM
6/1/08 12:00 AM
6/21/08 12:00 AM
7/11/08 12:00 AM
7/31/08 12:00 AM
8/20/08 12:00 AM
9/9/08 12:00 AM
9/29/08 12:00 AM
10/19/08 12:00 AM
11/8/08 12:00 AM
11/28/08 12:00 AM
12/18/08 12:00 AM
1/7/09 12:00 AM
1/27/09 12:00 AM
2/16/09 12:00 AM
3/8/09 12:00 AM
3/28/09 12:00 AM
4/17/09 12:00 AM
5/7/09 12:00 AM
5/27/09 12:00 AM
6/16/09 12:00 AM
7/6/09 12:00 AM
7/26/09 12:00 AM
8/15/09 12:00 AM
9/4/09 12:00 AM
9/24/09 12:00 AM
10/14/09 12:00 AM



Cosby Creek
pH
Stage, ft
Date
7
6
5
4
3
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
3/13/08 12:00 AM
4/2/08 12:00 AM
4/22/08 12:00 AM
5/12/08 12:00 AM
6/1/08 12:00 AM
6/21/08 12:00 AM
7/11/08 12:00 AM
7/31/08 12:00 AM
8/20/08 12:00 AM
9/9/08 12:00 AM
9/29/08 12:00 AM
10/19/08 12:00 AM
11/8/08 12:00 AM
11/28/08 12:00 AM
12/18/08 12:00 AM
1/7/09 12:00 AM
1/27/09 12:00 AM
2/16/09 12:00 AM
3/8/09 12:00 AM
3/28/09 12:00 AM
4/17/09 12:00 AM
5/7/09 12:00 AM
5/27/09 12:00 AM
6/16/09 12:00 AM
7/6/09 12:00 AM
7/26/09 12:00 AM
8/15/09 12:00 AM
9/4/09 12:00 AM
9/24/09 12:00 AM
10/14/09 12:00 AM

Appendix B: Supplementary Figures for Brook Trout *In Situ* Experiment During Episodes of Stream Acidification

Regression models of whole-body sodium concentrations (Figures 18-19) by preceding 24-h [H^+] (Body Na = 1.0006 - 0.0118·24-h H^+, n=96, p<0.0001, R2=0.36) and by Al_{TD} concentration (Body Na = 0.9952 - 0.0049·Al_{TD}, n=96, p<0.0001, R2=0.24) demonstrate reduced whole-body sodium concentrations were highly correlated with elevated H^+ and Al_{TD} concentrations. The lowest whole-body sodium concentrations occur when H^+ and Al_{TD} were both elevated (Figure 20).

Although no mortality was observed in this study, the loss of sodium indicated that fish were under physiological stress during the acid episodes. A 60-70% loss of sodium and chloride ions has been associated with the mortality of rainbow trout exposed to acid exposure (Wood and McDonald, 1982). Extrapolating the regression model outside of the observed sodium loss in this study, we would expect > 60% sodium loss, and therefore mortality, of adult brook trout when the 24-h mean pH is less than 4.3 during an episode of stream acidification.

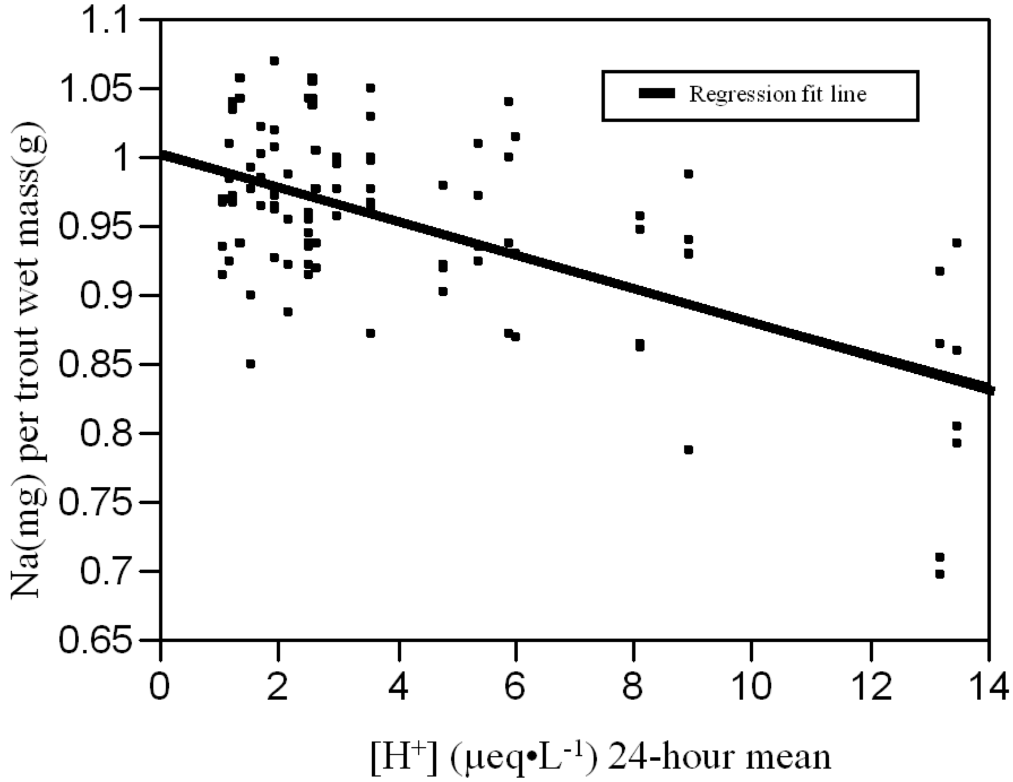


Figure 18: Least squares regression of whole-body sodium by hydrogen ion concentration.

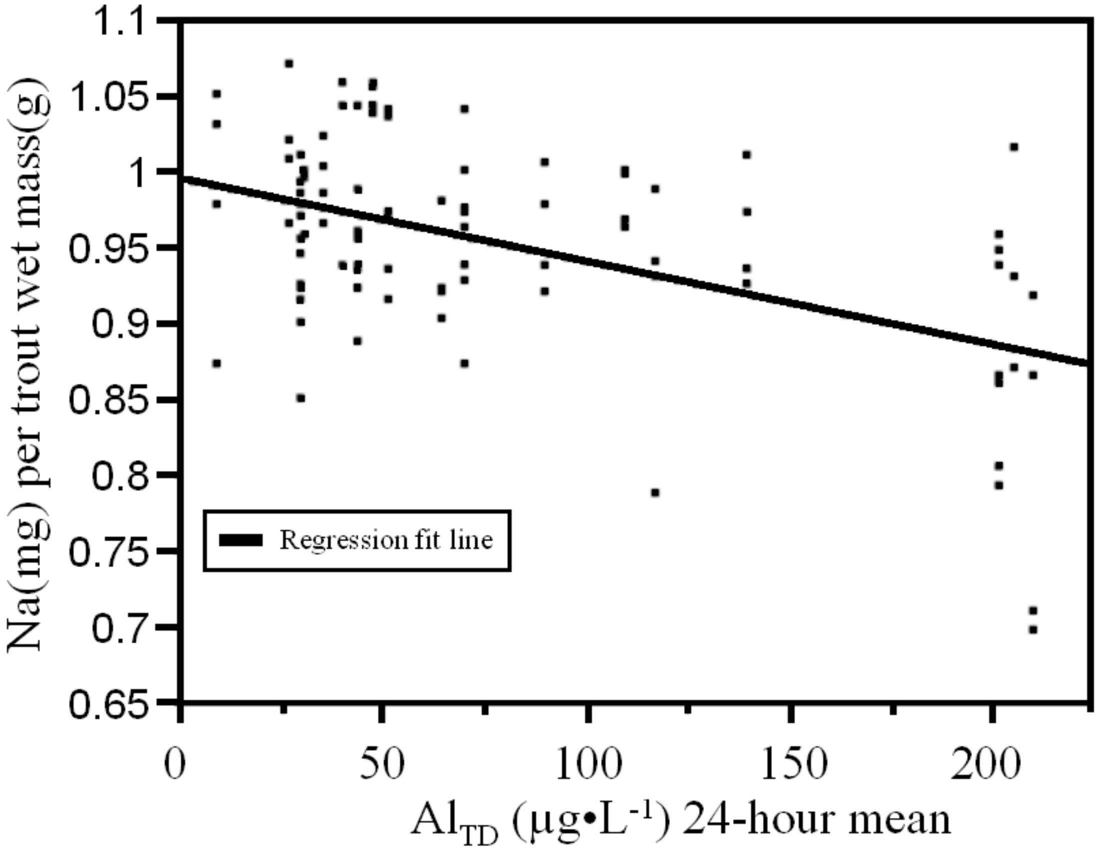


Figure 19: Least squares regression of whole-body sodium by Al$_{TD}$ concentration.

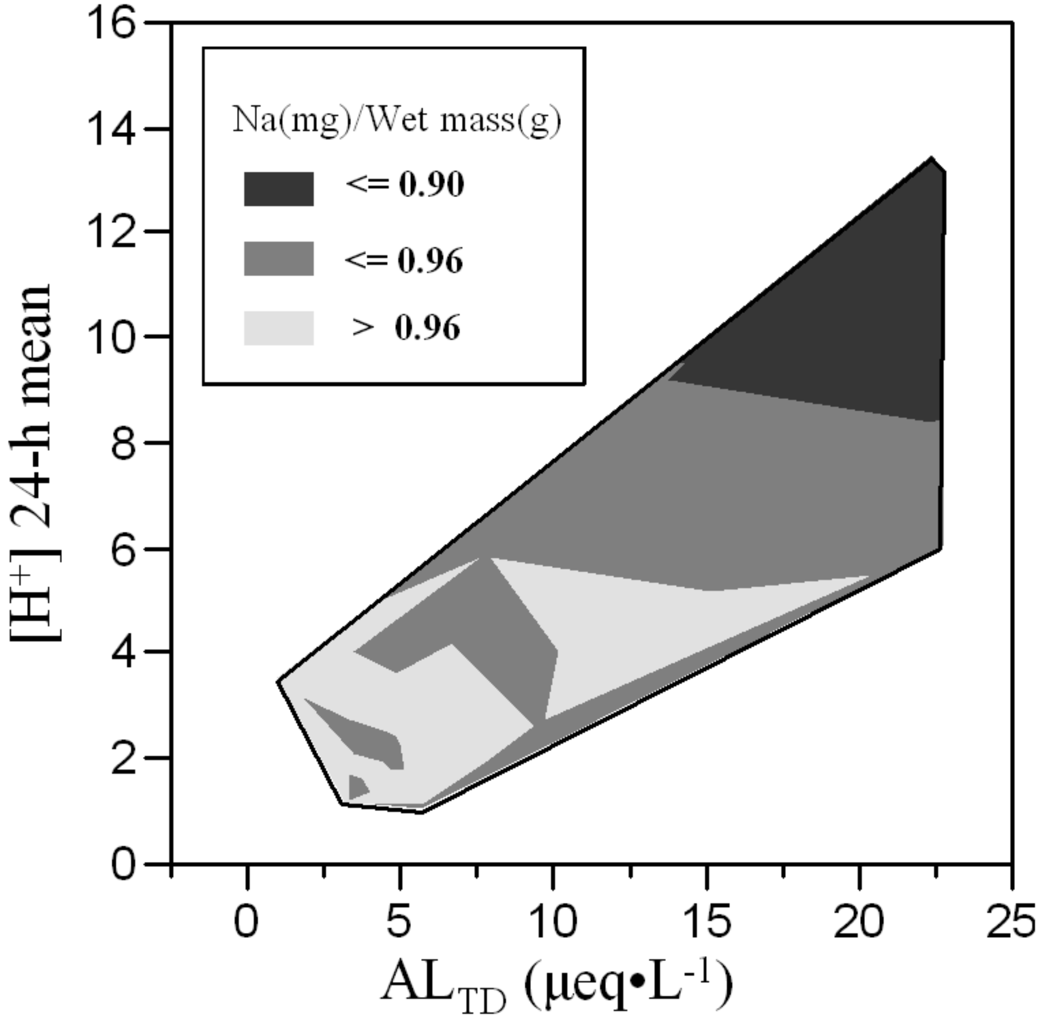


Figure 20: Contour plot for whole body sodium by Al_{TD} concentration and hydrogen ion concentration.

Appendix C: Supplementary Figures and Tables for Study of Chemical, Hydrological and Basin Factors Influencing Brook Trout

Linear Regression of K-factor and Collocated Site Characterization

A positively sloped linear regression of k-factor versus time, significant at the $\alpha=0.05$ level (Figure 21), indicates fish health was improving during the 20 year period. This may be associated with non-significant increases in pH and ANC concentrations during this time period.

Table 14 provides the average values of biotic, hydrologic, basin, and chemical factor values of 28 fish and water quality collocated sites. Seventeen of these sites were used for analysis in Chapter V. The sites used included the 11 allopatric brook trout sites and 5 fishless sites identified in Chapter V.

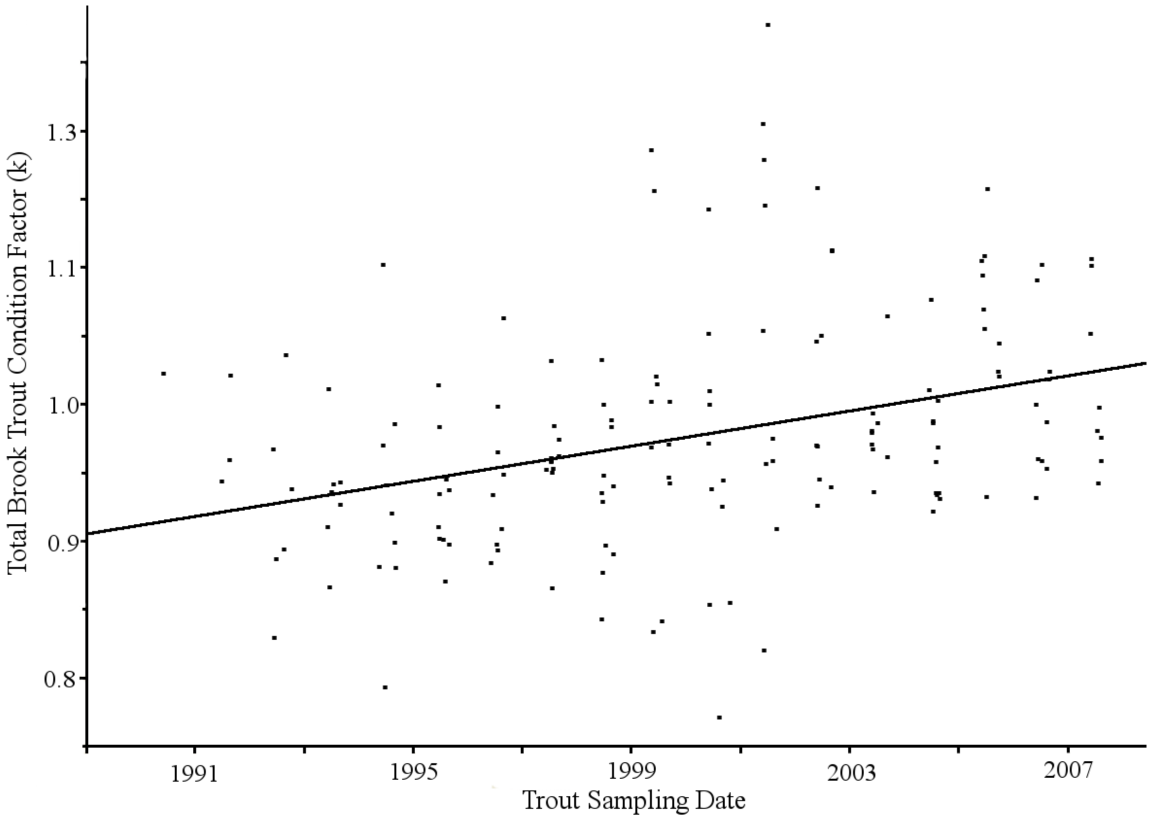


Figure 21: Linear regression (n=160, r^2=0.12) of total brook trout condition factor (K) versus sampling date. Model, intercept and independent variable significant (p<0.01).

Table 14: Average values of biotic, hydrologic, basin, and chemical factors of collocated sites.

Collocated Site	Fish Site	BKT Years	BKT TOT DENS
Bunches Creek	BUN-1	1990-2004,2006-2009	33.806
Cannon Creek	CAN-1		0.000
Cataloochee Creek (lower)	CAT-1	1997-1998,2000-2003,2008	0.045
Cataloochee (middle)	CAT-2	2003, 2008	0.094
Palmer Creek	CAT-4	1990-1992,1995,1997-1998,2000,2002-2003,2008	0.415
Cosby Creek	COS-2	1995-2009	17.134
Flat Creek	FLT-1	1992-2008	50.981
Hazel Creek (lower)	HAZ-1	1998, 2002	0.026
Hazel Creek (upper)	HAZ-3	1996-2002,2004-2006,2008-2009	22.033
Indian Camp Creek(lower)	ICC-2	1992-1995,1997-2009	12.314
Indian Camp Creek(upper)	ICC-3N	1994-1995,1997-2009	15.564
Lost Bottom Creek	LOB-0	1994-2005	8.373
Little River (lower)	LRV-0		0.000
Little River (middle)	LRV-1		0.000
Little River (upper)	LRV-2		0.000
Rock Creek (lower)	ROC-2	1993-2005	3.745
Rock Creek (upper)	ROC-7	1991-2009	6.728
Road Prong	RPR-5	1992-1993	7.912
Starkey Creek	STK-1	1990-2004	6.108
Thunderhead Prong	THD-C1		0.000
Walker Creek	WAL-1N	1998-1999,2004-2006,2008-2009	7.297
Bear Branch	BRB*		0.000
Cosby Creek	COS*		0.000
Shutts Prong	SHP*		0.000
Eagle Rocks Prong	ERP*		0.000
Ashe Camp Prong	ACB-3	2003-2005	16.448
Sams Creek	SAM-6	1990,1992-1999,2003-2004,2006-2008	14.355
Silers Creek	SIL-1	1992-2005	9.070
Porters Creek	POR*		0.000
Ramsey Prong	RAM*		0.000
Lost Bottom Creek (2)	LOB-1	1990-2005	12.929

Table 14: (continued).

Collocated Site	BKT YOY DENS	BKT ADT DENS	BKT K	Sympatric	BKT/RBT
Bunches Creek	11.650	22.243	0.993	No	∞
Cannon Creek	0.000	0.000		No	0.000
Cataloochee Creek (lower)	0.016	0.027	0.833	Yes	0.005
Cataloochee (middle)	0.050	0.042	1.000	Yes	0.011
Palmer Creek	0.190	0.218	0.933	Yes	0.043
Cosby Creek	8.392	8.982	1.044	No	∞
Flat Creek	21.391	29.868	0.958	No	∞
Hazel Creek (lower)	0.000	0.026	0.873	Yes	0.004
Hazel Creek (upper)	9.993	12.565	0.950	No	∞
Indian Camp Creek(lower)	6.175	6.124	0.978	Yes	61.821
Indian Camp Creek(upper)	4.803	10.923	0.994	No	∞
Lost Bottom Creek	2.937	5.890	0.931	Yes	1.457
Little River (lower)	0.000	0.000		No	0.000
Little River (middle)	0.000	0.000		No	0.000
Little River (upper)	0.000	0.000		No	0.000
Rock Creek (lower)	1.577	2.168	0.959	Yes	4.470
Rock Creek (upper)	2.588	4.138	1.006	No	∞
Road Prong	2.330	5.637	0.974	Yes	84.983
Starkey Creek	2.023	4.067	0.929	Yes	6.367
Thunderhead Prong	0.000	0.000		No	0.000
Walker Creek	4.432	2.784	0.980	Yes	1.354
Bear Branch				No	
Cosby Creek				No	
Shutts Prong	0.000	0.000		No	
Eagle Rocks Prong	0.000	0.000		No	
Ashe Camp Prong	9.237	7.500	0.861	No	
Sams Creek	3.721	10.757	0.946	No	
Silers Creek	3.101	5.912	0.921	No	
Porters Creek	0.000	0.000		No	
Ramsey Prong	0.000	0.000		No	
Lost Bottom Creek (2)	8.018	4.906	0.993	No	

Table 14: (continued).

Collocated Site	RBT Years	RBT YOY DENS	RBT ADT DENS	RBT TOT DENS
Bunches Creek		0.000	0.000	0.000
Cannon Creek	1995-2002, 2009	1.682	3.169	4.851
Cataloochee Creek (lower)	1990-2003,2008	5.169	3.852	8.798
Cataloochee (middle)	1990-2003,2008	4.145	4.185	8.172
Palmer Creek	1990-2003,2008	3.987	5.580	9.618
Cosby Creek		0.000	0.000	0.000
Flat Creek		0.000	0.000	0.000
Hazel Creek (lower)	1996-1999,2002	2.397	3.430	5.778
Hazel Creek (upper)		0.000	0.000	0.000
Indian Camp Creek(lower)	1995,2002-204,2006-2009	0.058	0.141	0.199
Indian Camp Creek(upper)		0.000	0.000	0.000
Lost Bottom Creek	1994-2005	0.793	4.940	5.746
Little River (lower)	1996-1998	0.047	0.434	0.488
Little River (middle)	1991-1994,1996-1999,1002-2003,2006-2007	2.434	2.657	5.146
Little River (upper)	1991-1999,2001-2003	4.203	4.450	8.652
Rock Creek (lower)	1993-2005	0.138	0.700	0.838
Rock Creek (upper)		0.000	0.000	0.000
Road Prong	1992-1993	0.000	0.093	0.093
Starkey Creek	1990,1992-2000	0.399	0.577	0.959
Thunderhead Prong	2000-2003,2005	6.317	6.058	12.409
Walker Creek	1998-1999,2004-2006,2008-2009	3.246	2.022	5.390
Bear Branch				
Cosby Creek				
Shutts Prong				
Eagle Rocks Prong				
Ashe Camp Prong				
Sams Creek				
Silers Creek				
Porters Creek				
Ramsey Prong				
Lost Bottom Creek (2)				

Table 14: (continued).

Collocated Site	Median Q	January Q	February Q	March Q	April Q	May Q
Bunches Creek	3.9	1.47	1.30	1.35	1.12	0.92
Cannon Creek	4.3	1.42	1.32	1.40	1.21	1.01
Cataloochee Creek (lower)	90.0	1.33	1.50	1.63	1.42	1.15
Cataloochee (middle)	83.5	1.38	1.35	1.40	1.28	1.07
Palmer Creek	31.1	1.40	1.33	1.37	1.22	1.02
Cosby Creek	4.2	1.28	1.25	1.34	1.27	1.11
Flat Creek	1.6	1.47	1.31	1.35	1.12	0.92
Hazel Creek (lower)	46.8	1.35	1.28	1.36	1.20	1.07
Hazel Creek (upper)	4.1	1.34	1.20	1.25	1.12	1.01
Indian Camp Creek(lower)	6.8	1.48	1.30	1.38	1.20	0.97
Indian Camp Creek(upper)	6.0	1.41	1.29	1.35	1.17	0.98
Lost Bottom Creek	7.4	1.41	1.31	1.36	1.19	0.99
Little River (lower)	198.5	1.57	1.55	1.73	1.44	1.05
Little River (middle)	102.0	1.58	1.31	1.35	1.22	1.02
Little River (upper)	91.5	1.51	1.29	1.34	1.21	1.04
Rock Creek (lower)	2.6	1.35	1.28	1.35	1.27	1.07
Rock Creek (upper)	2.4	1.29	1.27	1.34	1.26	1.08
Road Prong	7.3	1.46	1.30	1.34	1.13	0.93
Starkey Creek	2.1	1.35	1.24	1.30	1.20	1.09
Thunderhead Prong	8.5	1.33	1.56	1.37	1.26	1.03
Walker Creek	6.4	1.29	1.26	1.35	1.22	1.11
Bear Branch	0.6	1.19	1.39	1.43	1.38	1.15
Cosby Creek	0.9	1.14	1.18	1.28	1.26	1.10
Shutts Prong	4.2	1.41	1.36	1.54	1.24	1.07
Eagle Rocks Prong	9.3	1.51	1.35	1.26	1.05	1.02
Ashe Camp Prong	1.9	1.35	1.30	1.36	1.30	1.14
Sams Creek	2.4	1.33	1.20	1.27	1.15	1.05
Silers Creek	3.7	1.33	1.19	1.25	1.12	1.03
Porters Creek	7.5	1.44	1.31	1.26	1.12	1.14
Ramsey Prong	9.0	1.44	1.31	1.26	1.12	1.14
Lost Bottom Creek (2)	7.4	1.41	1.31	1.36	1.19	0.99

Table 14: (continued).

Collocated Site	June Q	July Q	August Q	September Q	October Q	November Q
Bunches Creek	0.96	0.79	0.68	0.66	0.61	0.89
Cannon Creek	1.01	0.81	0.68	0.62	0.58	0.80
Cataloochee Creek (lower)	0.86	0.72	0.67	0.61	0.50	0.63
Cataloochee (middle)	0.99	0.81	0.69	0.60	0.56	0.76
Palmer Creek	0.99	0.81	0.69	0.63	0.58	0.82
Cosby Creek	1.06	0.88	0.77	0.67	0.60	0.74
Flat Creek	0.96	0.80	0.68	0.66	0.62	0.88
Hazel Creek (lower)	1.00	0.88	0.77	0.68	0.57	0.75
Hazel Creek (upper)	1.00	0.88	0.79	0.78	0.65	0.88
Indian Camp Creek(lower)	0.97	0.78	0.66	0.61	0.57	0.84
Indian Camp Creek(upper)	0.99	0.82	0.70	0.66	0.61	0.85
Lost Bottom Creek	0.98	0.81	0.69	0.65	0.59	0.84
Little River (lower)	0.82	0.66	0.54	0.39	0.38	0.72
Little River (middle)	0.88	0.75	0.61	0.55	0.52	0.83
Little River (upper)	0.91	0.79	0.65	0.60	0.55	0.82
Rock Creek (lower)	1.04	0.85	0.73	0.64	0.58	0.75
Rock Creek (upper)	1.05	0.86	0.76	0.68	0.61	0.75
Road Prong	0.97	0.80	0.68	0.66	0.61	0.89
Starkey Creek	0.99	0.89	0.76	0.70	0.60	0.80
Thunderhead Prong	0.87	0.73	0.60	0.53	0.51	0.81
Walker Creek	1.03	0.91	0.79	0.69	0.58	0.73
Bear Branch	0.96	0.83	0.81	0.61	0.61	0.69
Cosby Creek	1.03	0.96	0.90	0.77	0.71	0.73
Shutts Prong	0.99	0.85	0.78	0.54	0.51	0.68
Eagle Rocks Prong	0.99	0.85	0.66	0.62	0.52	0.74
Ashe Camp Prong	0.97	0.83	0.73	0.63	0.57	0.75
Sams Creek	1.01	0.91	0.79	0.75	0.61	0.84
Silers Creek	1.01	0.90	0.80	0.77	0.63	0.87
Porters Creek	1.04	0.89	0.71	0.61	0.56	0.75
Ramsey Prong	1.04	0.89	0.71	0.61	0.56	0.75
Lost Bottom Creek (2)	0.98	0.81	0.69	0.65	0.59	0.84

Table 14: (continued).

Collocated Site	December Q	Reversals	X	Y	Site Elevation (m)	Basin Area (km2)
Bunches Creek	1.24	93.39	303519	3936291	1408	3.96
Cannon Creek	1.14	91.61	282773	3951124	767	5.01
Cataloochee Creek (lower)	0.98	116.78	312412	3949104	746	127.61
Cataloochee (middle)	1.11	96.72	312083	3946682	778	121.58
Palmer Creek	1.15	96.06	308153	3945403	861	37.56
Cosby Creek	1.02	90.06	301180	3957932	822	5.35
Flat Creek	1.24	90.50	302907	3936501	1476	1.39
Hazel Creek (lower)	1.09	103.22	258518	3932251	737	52.95
Hazel Creek (upper)	1.12	99.28	265793	3936501	1187	3.11
Indian Camp Creek(lower)	1.23	94.94	294115	3959322	683	7.78
Indian Camp Creek(upper)	1.17	93.28	294170	3957156	955	6.32
Lost Bottom Creek	1.17	93.61	305768	3945741	1009	8.46
Little River (lower)	1.16	114.33	254314	3950448	337	275.42
Little River (middle)	1.38	105.89	260584	3950895	520	125.98
Little River (upper)	1.29	105.67	264166	3949708	610	107.50
Rock Creek (lower)	1.10	91.06	300110	3959651	639	3.62
Rock Creek (upper)	1.04	90.61	299635	3957956	864	3.16
Road Prong	1.22	94.06	276285	3945394	1133	7.60
Starkey Creek	1.08	96.50	259021	3940788	993	2.43
Thunderhead Prong	1.38	102.00	258006	3943831	641	11.38
Walker Creek	1.04	99.22	261242	3934399	889	7.47
Bear Branch	0.96	85.00	302487	3942487	1681	0.57
Cosby Creek	0.94	86.50	302560	3957549	1168	0.66
Shutts Prong	1.03	85.67	282956	3948665	1015	3.41
Eagle Rocks Prong	1.44	98.28	290104	3951955	975	10.47
Ashe Camp Prong	1.07	94.11	267024	3942543	1049	1.65
Sams Creek	1.08	96.00	259523	3940321	1075	2.29
Silers Creek	1.10	98.06	267411	3941775	1064	3.35
Porters Creek	1.19	95.28	283167.68	3949854	847	8.56
Ramsey Prong	1.19	93.61	289234.04	3953655	840	10.30
Lost Bottom Creek (2)	1.17	93.61	305768	3945741	1009	8.46

Table 14: (continued).

Collocated Site	Mean Basin Elevation (m)	Stream Order	Channel Slope	Longest Flow Path (km)	10%-85% Elevation Dif.	Downstream Fish Barriers
Bunches Creek	1587	2	7.52	3.90	219.95	Yes
Cannon Creek	1334	2	21.72	4.87	793.64	Yes
Cataloochee Creek (lower)	1214	4	2.82	19.69	416.52	No
Cataloochee (middle)	1226	4	3.52	16.69	440.60	No
Palmer Creek	1332	4	6.30	10.29	485.91	No
Cosby Creek	1228	3	17.99	3.57	481.94	No
Flat Creek	1566	1	5.17	2.91	112.93	Yes
Hazel Creek (lower)	1210	4	5.07	13.47	511.87	No
Hazel Creek (upper)	1444	2	14.11	3.30	349.30	Yes
Indian Camp Creek(lower)	1363	3	15.64	6.30	738.56	No
Indian Camp Creek(upper)	1464	3	20.48	3.80	584.07	No
Lost Bottom Creek	1422	3	10.30	6.55	505.80	Yes
Little River (lower)	979	5	1.89	44.15	626.18	No
Little River (middle)	1133	4	2.69	28.83	582.46	Yes
Little River (upper)	1188	4	3.66	22.10	606.79	Yes
Rock Creek (lower)	1250	2	18.60	5.41	755.14	No
Rock Creek (upper)	1323	2	24.27	3.46	629.84	No
Road Prong	1555	3	12.61	5.18	489.55	Yes
Starkey Creek	1274	2	17.22	2.52	325.67	Yes
Thunderhead Prong	1159	3	13.59	6.83	696.62	No
Walker Creek	1186	2	7.17	7.06	379.69	No
Bear Branch	1721	1	23.56	0.53	94.00	No
Cosby Creek	1332	1	51.44	0.66	255.00	No
Shutts Prong	1382	2	16.25	3.17	386.00	No
Eagle Rocks Prong	1445	3	12.45	4.88	455.5	Yes
Ashe Camp Prong	1253	2	17.60	2.08	274.56	Yes
Sams Creek	1332	2	18.03	2.32	313.70	Yes
Silers Creek	1338	2	13.00	3.75	365.41	Yes
Porters Creek	1292	3	12.17	5.60	511.00	No
Ramsey Prong	1418	3	12.80	8.37	803.35	No
Lost Bottom Creek (2)	1422	3	10.30	6.55	505.80	Yes

Table 14: (continued).

Collocated Site	Anakeesta (km2)	Anakeesta (%)	Mean Slope (%)	Soil pH	Soil CEC	Soil Organic
Bunches Creek	0.00	0	20.0	4.55	6.77	9.02
Cannon Creek	0.03	1	30.6	4.21	4.63	5.89
Cataloochee Creek (lower)	0.00	0	25.2	4.57	4.77	6.30
Cataloochee (middle)	0.00	0	25.3	4.57	4.81	6.35
Palmer Creek	0.00	0	26.3	4.60	5.03	6.67
Cosby Creek	0.00	0	31.9	4.47	4.29	5.12
Flat Creek	0.00	0	16.7	4.49	6.29	9.26
Hazel Creek (lower)	4.68	9	26.8	4.57	4.69	6.05
Hazel Creek (upper)	0.66	21	25.7	4.55	5.99	8.12
Indian Camp Creek(lower)	0.00	0	29.2	4.34	5.25	5.40
Indian Camp Creek(upper)	0.00	0	30.4	4.28	5.33	4.79
Lost Bottom Creek	0.00	0	26.4	4.60	5.33	6.99
Little River (lower)	42.32	15	25.7	4.58	3.99	5.23
Little River (middle)	21.88	17	25.5	4.51	4.39	5.71
Little River (upper)	21.90	20	25.8	4.49	4.52	5.74
Rock Creek (lower)	0.00	0	30.0	4.39	5.00	6.41
Rock Creek (upper)	0.00	0	32.4	4.36	4.85	5.71
Road Prong	1.15	15	25.4	4.15	5.77	5.53
Starkey Creek	1.75	72	30.9	4.38	4.68	7.29
Thunderhead Prong	3.96	35	28.8	4.48	4.55	7.11
Walker Creek	0.97	13	25.8	4.53	4.60	5.87
Bear Branch	0.00	0	20.7	4.50	6.37	9.19
Cosby Creek	0.00	0	30.1	4.26	5.33	4.20
Shutts Prong	2.04	60	37.9	4.34	3.64	10.58
Eagle Rocks Prong	1.17	11	30.5	4.33	4.13	8.59
Ashe Camp Prong	1.64	0.99	27.5	4.64	3.70	5.35
Sams Creek	0.00	0.00	26.0	4.31	6.24	5.39
Silers Creek	0.44	0.13	26.9	4.35	5.11	5.64
Porters Creek	4.30	0.50	34.9	4.42	3.96	9.01
Ramsey Prong	0.00	0.00	25.1	4.38	4.82	4.69
Lost Bottom Creek (2)	0.00	0.00	26.4	4.60	5.33	6.99

Table 14: (continued).

Collocated Site	Soil Ksat	Soil Depth	Soil Slope	SAMF	HEMF	LEMF
Bunches Creek	4.05	137.08	39.72	20.00%	76.09%	0.77%
Cannon Creek	8.98	63.95	57.08	19.83%	42.57%	15.60%
Cataloochee Creek (lower)	2.27	114.10	57.17	4.76%	38.33%	39.47%
Cataloochee (middle)	2.30	114.67	57.07	5.00%	40.13%	39.06%
Palmer Creek	2.67	115.50	57.52	7.35%	40.99%	32.20%
Cosby Creek	4.08	68.99	62.98	4.83%	40.15%	41.37%
Flat Creek	5.05	126.06	31.20	14.85%	83.47%	0.00%
Hazel Creek (lower)	2.00	117.23	57.79	2.40%	29.41%	62.86%
Hazel Creek (upper)	3.01	135.58	62.09	20.00%	58.98%	19.00%
Indian Camp Creek(lower)	4.53	74.96	56.63	21.01%	43.69%	28.22%
Indian Camp Creek(upper)	4.92	67.29	60.41	25.86%	52.89%	15.44%
Lost Bottom Creek	3.22	112.34	56.07	22.02%	13.24%	52.21%
Little River (lower)	3.09	79.79	53.39	4.09%	15.56%	50.34%
Little River (middle)	3.39	80.89	53.96	8.67%	21.86%	43.64%
Little River (upper)	3.55	79.99	54.12	10.17%	25.56%	40.79%
Rock Creek (lower)	4.31	79.87	56.88	18.11%	36.89%	35.30%
Rock Creek (upper)	4.49	71.35	62.54	20.74%	42.25%	26.01%
Road Prong	5.12	71.20	57.43	72.37%	26.49%	0.00%
Starkey Creek	3.60	78.33	60.08	0.04%	69.63%	18.89%
Thunderhead Prong	3.59	84.31	55.70	0.11%	25.63%	56.26%
Walker Creek	1.70	114.11	55.61	0.00%	29.76%	67.12%
Bear Branch	5.03	124.95	21.12	63.80%	36.20%	0.00%
Cosby Creek	4.54	62.80	62.42	22.11%	75.75%	0.00%
Shutts Prong	6.42	85.70	61.43	29.47%	62.52%	1.24%
Eagle Rocks Prong	4.78	80.42	60.71	50.77%	37.82%	4.38%
Ashe Camp Prong	2.98	75.18	57.90	0.00%	67.96%	16.19%
Sams Creek	4.13	82.79	62.34	0.40%	69.26%	25.68%
Silers Creek	4.17	73.72	57.30	7.31%	57.61%	27.41%
Porters Creek	4.90	85.51	59.67	20.36%	45.60%	14.07%
Ramsey Prong	4.18	67.76	57.78	38.50%	41.75%	9.44%
Lost Bottom Creek (2)	3.22	112.34	56.07	22.02%	13.24%	52.21%

Table 14: (continued).

Collocated Site	LEXF	Shrub	pH	ANC	Cl	NO3
Bunches Creek	0.00%	0.70%	6.28	40.80	17.08	24.36
Cannon Creek	13.86%	8.13%	5.94	16.25	15.19	21.83
Cataloochee Creek (lower)	13.25%	1.79%	6.63	82.41	15.54	10.02
Cataloochee (middle)	11.51%	1.88%	6.61	81.09	14.21	10.08
Palmer Creek	13.88%	4.42%	6.53	69.07	14.47	9.22
Cosby Creek	8.58%	5.06%	6.30	35.82	15.94	38.90
Flat Creek	0.00%	0.00%	6.41	49.81	16.99	25.62
Hazel Creek (lower)	4.41%	0.62%	6.44	55.43	15.01	6.92
Hazel Creek (upper)	0.65%	0.00%	6.17	28.29	16.37	24.84
Indian Camp Creek(lower)	3.91%	3.08%	6.22	36.49	14.50	24.44
Indian Camp Creek(upper)	1.90%	3.79%	6.06	19.27	15.54	35.19
Lost Bottom Creek	1.66%	10.70%	6.41	48.56	13.56	8.41
Little River (lower)	23.28%	5.33%	6.67	103.02	15.05	8.80
Little River (middle)	13.86%	9.79%	6.55	82.63	15.93	9.15
Little River (upper)	10.18%	10.91%	6.44	66.07	18.72	9.56
Rock Creek (lower)	0.19%	9.42%	6.08	37.72	13.02	21.26
Rock Creek (upper)	0.22%	10.79%	5.84	11.26	15.01	33.96
Road Prong	0.00%	1.00%	6.13	28.56	14.90	35.22
Starkey Creek	0.00%	11.43%	6.09	20.87	14.98	23.37
Thunderhead Prong	11.18%	6.78%	6.25	33.22	15.67	15.07
Walker Creek	2.94%	0.18%	6.49	72.01	15.15	3.65
Bear Branch	0.00%	0.00%	5.09	-3.64	17.07	48.77
Cosby Creek	1.98%	0.16%	6.26	38.45	17.98	30.94
Shutts Prong	0.00%	2.94%	5.58	4.56	16.94	30.75
Eagle Rocks Prong	0.00%	5.22%	5.39	-0.30	11.90	49.60
Ashe Camp Prong	0.00%	10.58%	6.40	51.40	17.10	12.34
Sams Creek	0.00%	4.66%	6.10	18.74	16.50	19.16
Silers Creek	0.00%	6.41%	6.28	36.35	16.89	14.73
Porters Creek	6.89%	2.89%	5.72	5.39	15.36	27.17
Ramsey Prong	6.52%	2.79%	5.55	5.10	12.23	34.87
Lost Bottom Creek (2)	1.66%	10.70%	6.41	48.56	13.56	8.41

Table 14: (continued).

Collocated Site	SO4	Anions	Na	NH4	K	Mg
Bunches Creek	17.06	58.50	38.01	0.65	10.47	22.15
Cannon Creek	42.98	80.00	25.11	0.95	9.12	21.55
Cataloochee Creek (lower)	21.92	47.49	48.47	0.18	14.84	26.72
Cataloochee (middle)	21.77	46.06	48.59	0.21	14.28	26.79
Palmer Creek	18.75	42.45	42.52	0.77	12.95	19.90
Cosby Creek	46.96	101.81	34.75	0.68	9.74	36.26
Flat Creek	11.60	54.21	39.79	0.56	12.41	22.82
Hazel Creek (lower)	16.93	38.86	37.78	0.04	11.04	20.43
Hazel Creek (upper)	17.45	58.66	32.78	0.20	10.38	18.96
Indian Camp Creek(lower)	44.81	81.71	35.93	0.14	12.57	21.23
Indian Camp Creek(upper)	51.81	102.53	29.28	0.43	13.65	20.79
Lost Bottom Creek	21.48	43.45	35.99	0.95	11.43	17.13
Little River (lower)	36.59	60.44	40.76	0.66	12.97	40.42
Little River (middle)	33.87	58.95	38.56	0.35	12.08	29.10
Little River (upper)	31.61	59.89	36.80	2.23	12.51	26.65
Rock Creek (lower)	36.21	70.49	37.69	0.43	10.54	23.48
Rock Creek (upper)	51.37	98.63	31.00	0.22	9.17	26.42
Road Prong	47.12	96.01	27.97	0.45	9.49	29.29
Starkey Creek	41.87	80.22	26.34	0.83	6.51	23.37
Thunderhead Prong	31.62	60.76	33.83	0.32	10.20	19.46
Walker Creek	15.53	34.34	39.89	0.27	11.31	24.14
Bear Branch	26.30	92.14	30.58	0.25	7.96	24.14
Cosby Creek	49.34	98.27	34.75	0.16	9.16	35.81
Shutts Prong	90.29	137.98	23.94	0.99	5.17	48.53
Eagle Rocks Prong	50.90	110.81	27.30	0.74	11.52	31.50
Ashe Camp Prong	35.48	64.55	36.25	0.45	9.38	25.81
Sams Creek	27.86	61.93	29.55	0.45	9.41	17.28
Silers Creek	23.57	54.04	33.12	0.72	10.02	19.32
Porters Creek	75.81	118.08	27.69	0.24	4.44	42.08
Ramsey Prong	40.34	87.44	28.46	0.94	11.63	18.36
Lost Bottom Creek (2)	21.48	43.45	35.99	0.95	11.43	17.13

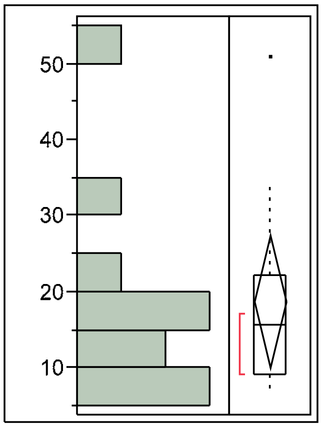
Table 14: (continued).

Collocated Site	Ca	Cations	Al	RSE TOT BKT	RSE ADT BKT	RSE YOY BKT
Bunches Creek	44.80	116.10	0.05	9.06%	6.55%	14.51%
Cannon Creek	50.40	107.12	0.09	0.00%	0.00%	0.00%
Cataloochee Creek (lower)	53.32	143.52	0.03	39.02%	21.80%	52.60%
Cataloochee (middle)	53.61	143.48	0.03	91.86%	39.05%	94.79%
Palmer Creek	44.24	113.08	0.03	49.16%	28.04%	49.53%
Cosby Creek	70.23	151.67	0.03	17.97%	4.84%	36.45%
Flat Creek	44.43	120.02	0.05	5.59%	3.31%	9.97%
Hazel Creek (lower)	40.30	109.59	0.04	68.28%	68.28%	0.00%
Hazel Creek (upper)	36.82	99.14	0.03	10.69%	7.57%	16.74%
Indian Camp Creek(lower)	70.16	140.04	0.03	7.93%	4.91%	14.47%
Indian Camp Creek(upper)	74.94	139.09	0.06	5.82%	4.21%	13.94%
Lost Bottom Creek	39.45	104.96	0.03	8.68%	7.98%	26.52%
Little River (lower)	84.34	179.15	0.02	0.00%	0.00%	0.00%
Little River (middle)	78.21	158.32	0.06	0.00%	0.00%	0.00%
Little River (upper)	66.24	144.43	0.13	0.00%	0.00%	0.00%
Rock Creek (lower)	54.87	126.30	0.05	23.71%	11.20%	37.25%
Rock Creek (upper)	56.93	122.15	0.05	12.44%	7.01%	27.62%
Road Prong	74.24	139.64	0.06	7.72%	6.58%	17.66%
Starkey Creek	55.44	109.82	0.02	20.28%	16.73%	38.70%
Thunderhead Prong	47.99	108.92	0.07	0.00%	0.00%	0.00%
Walker Creek	46.73	122.34	0.03	18.32%	7.67%	57.64%
Bear Branch	41.81	104.74	0.12	0.00%	0.00%	0.00%
Cosby Creek	67.56	147.44	0.07	0.00%	0.00%	0.00%
Shutts Prong	73.25	151.88	0.10	0.00%	0.00%	0.00%
Eagle Rocks Prong	53.50	120.90	0.07	0.00%	0.00%	0.00%
Ashe Camp Prong	57.66	129.55		7.08%	7.12%	29.28%
Sams Creek	44.03	98.20		11.49%	8.39%	22.22%
Silers Creek	42.01	103.91		8.18%	5.08%	18.54%
Porters Creek	60.70	135.19		0.00%	0.00%	0.00%
Ramsey Prong	44.61	103.99	0.09	0.00%	0.00%	0.00%
Lost Bottom Creek (2)	39.45	104.96	0.03	9.37%	2.93%	14.41%

Distribution of Brook Trout Densities

The distribution of brook trout densities in the 11 allopatric brook trout sites is illustrated below.

Distribution and simple statistics of total brook trout of 11 sites with brook trout populations in Chapter 5



Quantiles

100.0%	maximum	50.981
99.5%		50.981
97.5%		50.981
90.0%		47.546
75.0%	quartile	22.033
50.0%	median	15.564
25.0%	quartile	9.070
10.0%		6.964
2.5%		6.728
0.5%		6.728
0.0%	minimum	6.728

Moments

Mean	18.814583
Std Dev	13.050785
Std Err Mean	3.9349598
Upper 95% Mean	27.58222
Lower 95% Mean	10.046946
N	11

Spearman Bivariate Correlations of Brook Trout Densities with Factor Variables

Following is a table demonstrating the significant spearman bivariate correlations of brook trout densities with independent variables (Table 8) in these sites. Hydrologic factors were not significantly correlated with brook trout densities ($p<0.05$).

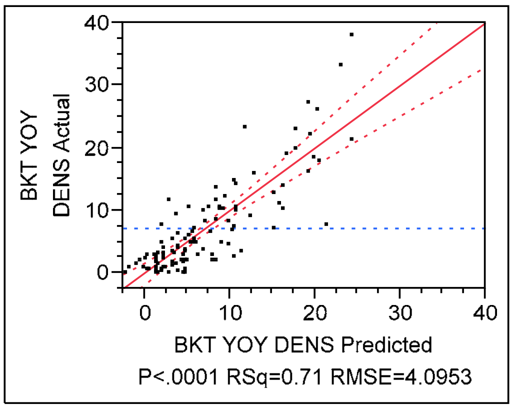
Significant Spearman bivariate correlations of brook trout densities with independent variable (Table 8) in the 11 collocated sites with brook trout populations.

	Independent Variable	Spearman ρ	Probability > |ρ|
Basin Factors	10%-85% Elevation Dif.	-0.7455	0.0085
	HEMF	0.6636	0.0260
	Shrub	-0.6743	0.0229
Chemistry Factors	AVG Na	0.6545	0.0289
	AVG SO_4	-0.6364	0.0353
	AVG Cl	0.6182	0.0426

Chapter V Linear Regression Models

Multiple linear regression of YOY brook trout density by hydrologic, chemical, and basin variables.

Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.71233
RSquare Adj	0.698499
Root Mean Square Error	4.095298
Mean of Response	7.194394
Observations (or Sum Wgts)	110

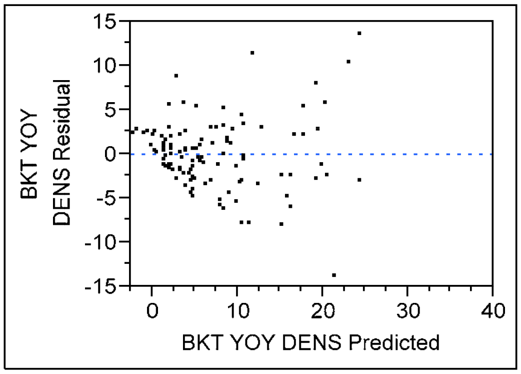
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	4319.0665	863.813	51.5049
Error	104	1744.2321	16.771	**Prob > F**
C. Total	109	6063.2986		<.0001*

Parameter Estimates

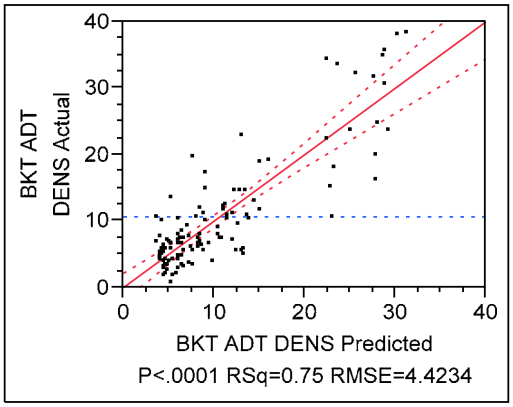
Term	Estimate	Std Error	t Ratio	Prob>|t|	VIF
Intercept	-189.7333	26.20039	-7.24	<.0001*	.
Median Q Preceding WY	-1.18551	0.207592	-5.71	<.0001*	1.4696087
Soi Organic	4.6211264	0.465084	9.94	<.0001*	3.2122904
AVG pH	26.935134	4.029377	6.68	<.0001*	3.332506
1YR SO4	0.4173807	0.075898	5.50	<.0001*	8.6658565
1YR Mg	-0.331855	0.090979	-3.65	0.0004*	2.3792259

Residual by Predicted Plot



<u>Multiple linear regression of adult brook trout density by hydrologic, chemical, and basin variables.</u>

Whole Model
Actual by Predicted Plot



Summary of Fit

RSquare	0.750587
RSquare Adj	0.739453
Root Mean Square Error	4.423422
Mean of Response	10.55814
Observations (or Sum Wgts)	118

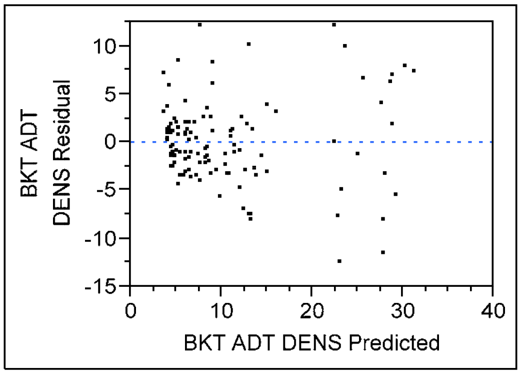
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	6595.0388	1319.01	67.4110
Error	112	2191.4662	19.57	**Prob > F**
C. Total	117	8786.5050		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|	VIF
Intercept	-39.60155	16.18841	-2.45	0.0160*	.
Reversals	-0.551951	0.153908	-3.59	0.0005*	1.295579
Soi Organic	3.5616046	0.36465	9.77	<.0001*	1.7724488
AVG Cl	3.5332038	0.417714	8.46	<.0001*	1.1924396
AVG K	1.6390272	0.318022	5.15	<.0001*	1.0984995
1YR Ca	0.1144841	0.03167	3.61	0.0005*	1.8088342

Residual by Predicted Plot



Appendix D: Block Designed Watersheds

In December 2007 and January 2008, Dr. John Schwartz, Steve Moore, Matt Kulp, and I discussed that a block design approach should be employed to determine differences in water quality from natural processes with respect to watershed size, elevation, and geology (presence or absence of sulfidic shale). After an initial ArcGIS analysis was employed to identify potential sites, we collaboratively selected sites for study. A proposal, included as part of the 2008 and 2009 annual contracts (Cooperative Agreement No.: 1443-CA-5460-98-006), entitled "2008/2009 Great Smoky Mountains National Park Water Quality Proposal: Episodic Stream Acidification in the Great Smoky Mountain National Park", detailing the block design, methodology, and site selection, was accepted by Nancy Finley, Steve Moore, Matt Kulp of the GRSM in early 2009. This project also met contractual obligations of the U.S. EPA agreement EM-83298901-1, through which it was primarily funded.

In January of 2009, a comprehensive ArcGIS analysis of block designed watersheds in the GRSM was conducted by Joseph Parker and me (Block-Designed Watersheds in the Great Smoky Mountains National Park). The scope of this report provided detailed information of block-designed basins in the GRSM.

Symmetric block design

Block	**Basin Area**	**Elevation**	**Anakeesta**
1	< 10 km^2	< 1000 m	>10%
2	< 10 km^2	> 1000 m	>10%
3	< 10 km^2	< 1000 m	None
4	< 10 km^2	> 1000 m	None
5	10 km^2 – 20 km^2	< 1000 m	>10%
6	10 km^2 – 20 km^2	> 1000 m	>10%
7	10 km^2 – 20 km^2	< 1000 m	None
8	10 km^2 – 20 km^2	> 1000 m	None

ArcGIS 9.2 was utilized to create watersheds for each block unit throughout the GRSM. The most current data, obtained from NPS personnel Benjamin Zank and Matt Kulp, is incorporated into GIS analyses. ArcHdyro tools and Spatial Analyst tools were used to delineate watersheds. The Spatial Analyst Zonal Statistics Tool was utilized to characterize block parameters for watersheds in the study. Spatial Statistics Calculate area tool was used to determine watershed areas.

A total of 556 watersheds were identified and created to represent the 8 block units in this GIS research. The total area of these watersheds is 2757 km2 which is greater than the actual area of the GRSM (2067 km2). This results because some of the watersheds from different block units overlap with other watersheds in other block units. The actual area of the union of all the block watersheds is 1582 km^2 which represents 77% of the total GRSM area. The areas not represented by these watersheds includes watersheds with 1)areas exceeding 20 km^2, 2) areas greater than 10% outside the GRSM boundary, 3) areas less than 1 km^2 which do no drain into other block watersheds, and 4) watersheds with 0% < Anakeesta % area < 10%.

Number of watersheds and total area of block units in the GRSM

Block	# of Watersheds	Area (km2)	% Park Area
1	43	191.16	9.25%
2	47	212.37	10.28%
3	261	955.07	46.21%
4	137	382.85	18.53%
5	16	236.13	11.43%
6	9	120.46	5.83%
7	33	526.85	25.49%
8	10	132.57	6.41%

General statistics of block units for area, Anakeesta and mean elevation

		Area(km2)	Anakeesta(km2)	% Anakeesta	Mean Elevation[2]
Block 1	Mean=	4.45	1.76	40.90%	1092
	Median=	3.03	1.12	34.25%	1172
	Stdev=	3.22	1.56	25.01%	225
Block 2	Mean=	4.52	1.80	42.52%	1369
	Median=	3.49	1.32	34.63%	1360
	Stdev=	3.01	1.68	26.25%	101
Block 3	Mean=	3.66	0.00	0.00%	916
	Median=	2.82	0.00	0.00%	935
	Stdev=	2.62	0.00	0.00%	245
Block 4	Mean=	2.79	0.00	0.00%	1323
	Median=	1.87	0.00	0.00%	1304
	Stdev=	2.26	0.00	0.00%	99
Block 5	Mean=	14.76	5.07	34.07%	1227
	Median=	13.72	3.99	31.61%	1289
	Stdev=	3.54	3.26	18.45%	156
Block 6	Mean=	13.38	4.61	31.47%	1409
	Median=	12.37	2.43	19.62%	1391
	Stdev=	3.58	4.64	26.59%	62
Block 7	Mean=	15.97	0.00	0.00%	1071
	Median=	16.47	0.00	0.00%	1041
	Stdev=	3.61	0.00	0.00%	281
Block 8	Mean=	13.26	0.00	0.00%	1429
	Median=	11.84	0.00	0.00%	1438
	Stdev=	3.60	0.00	0.00%	49

[2] Mean elevation is the average elevation of each watershed, not the elevation of the outlet.

Matrix: overlapping (shared areas) block watersheds

<u>1</u>	89.1 km^2 **1**:47% **2**:42%	0	4.1 km^2 **1**:2% **4**:1%	128.3 km^2 **1**:67% **5**:54%	0	0	0
1:21 **2**:25	**<u>2</u>**	0	0	122.1 km^2 **2**:57% **5**:52%	93.4 km^2 **2**:44% **6**:78%	0	0
0	0	**<u>3</u>**	211.2 km^2 **3**:22% **4**:55%	13.2 km^2 **3**:1% **5**:6%	0	322.2 km^2 **3**:34% **7**:61%	9.8 km^2 **3**:1% **8**:7%
1:1 **4**:1	0	**3**:78 **4**:99	**<u>4</u>**	9.4 km^2 **4**:2% **5**:4%	0	189.1 km^2 **4**:49% **7**:36%	108.9 km^2 **4**:28% **8**:82%
1:21 **5**:13	**2**:28 **5**:15	**3**:5 **5**:3	**4**:5 **5**:3	**<u>5</u>**	62.5 km^2 **5**:26% **6**:52%	0	0
0	**2**:13 **6**:9	0	0	**5**:5 **6**:5	**<u>6</u>**	0	0
0	0	**3**:67 **7**:27	**4**:57 **7**:25	0	0	**<u>7</u>**	112.2 km^2 **7**:21% **8**:85%
0	0	**3**:1 **8**:1	**4**:21 **8**:9	0	0	**7**:9 **8**:9	**<u>8</u>**

	Shared area; % shared area for each block (**block**:%)
-	Block`
	Number of watersheds overlapping (shared area) for each block (**block**:#)

Vita

Keil J. Neff graduated from Vanderbilt University with a Bachelor of Science in Engineering Science and Anthropology in May of 1997. In the subsequent eight years, he primarily worked as an archeological/osteological researcher in the southeastern United States and as a teacher (English instructor in South Korea for one year, and as a high school Mathematics teacher for four years). He began graduate studies in the Department of Civil and Environmental Engineering at the University of Tennessee in the fall of 2005, where he focused in Water Resources completing a Master of Science in Environmental Engineering in August of 2007. He was married to Laura Leigh Neff in 2003. In 2007, they had a son, Blaise Samson. After many laborious years conducting research, Keil earned a Ph.D. degree in Civil Engineering in December of 2010.